Method for achieving a pathogen resistance in plants

The invention relates to methods for generating or increasing a pathogen resistance in plants by expression, preferably pathogen-inducible expression, of a sucrose isomerase.

Palatinose (isomaltulose) and trehalulose are produced on a large scale from sucrose by an enzymatic rearrangement reaction using immobilized bacterial cells. The α1->β2-glycosidic bond, which exists between the monosaccharides of the disaccharide sucrose, is isomerized, in the case of palatinose to an α1->α6 bond or, in the case of trehalulose an α1->α1 bond. This rearrangement of sucrose to give the two noncariogenic disaccharides is catalyzed by the bacterial enzyme sucrose isomerase, also referred to as sucrose mutase. Suitable sequences are described for example in WO 95/20047 (US 5,786,140; US 5,985,622).

Also described are sucrose isomerases from Erwinia rhapontici (palI gene, GenBank Acc. No.: AF279281; Börnke et al. (2001) J Bacteriol 183(8):2425-2430) and Klebsiella sp. Strain LX3 (GenBank Acc. No.: AY040843; Zhang et al. (2002) Appl Environ Microbiol (68):2676-2682).

WO 01/59136 describes methods for the direct production of noncariogenic sugars directly in transgenic plants which comprise recombinant nucleic acid molecules encoding proteins with the enzymatic activity of a sucrose isomerase. Described are expression constructs for said sucrose isomerase for expression in plants, and the transgenic plants transformed therewith.

WO 01/59135 describes methods for influencing the pollen development using anther-, tapetum- or pollen-specifically expressed sucrose isomerases. However, the constitutive expression of sucrose isomerase in plants has an adverse effect on plant growth (Börnke F et al. (2002) Planta 214:356-364).

The aim of plant biotechnology work is the generation of plants with advantageous novel properties, for example for increasing agricultural productivity, increasing the quality in the case of foodstuffs, or for producing specific chemicals or pharmaceuticals. The plants' natural defense mechanisms against pathogens are frequently insufficient. Fungal diseases alone result in annual yield losses of many billions of US$. The introduction of foreign genes from plants, animals or microbial sources can increase the defenses. Examples are the protection of tobacco against feeding damage by insects by expressing *Bacillus*

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thuringiensis endotoxins under the control of the 35S CaMV promoter (Vaeck et al. (1987) Nature 328:33-37) or the protection of tobacco against fungal infection by expressing a bean chitinase under the control of the CaMV promoter (Broglie et al. (1991) Science 254:1194-1197). However, most of the approaches described only offer resistance to a single pathogen or a narrow spectrum of pathogens.

Only a few approaches exist which impart a resistance to a broader spectrum of pathogens, in particular fungal pathogens, to plants. Systemic acquired resistance (SAR) - a defense mechanism in a variety of plant/pathogen interactions - can be conferred by the application of endogenous messenger substances such as jasmonic acid (JA) or salicylic acid (SA) (Ward, et al. (1991) Plant Cell 3:1085-1094; Uknes, et al. (1992) Plant Cell 4(6):645-656). Similar effects can also be achieved by synthetic compounds such as 2,6-dichloroisonicotinic acid (INA) or S-methyl benzo(1,2,3)thiadiazole-7-thiocarboxylate (BTH; Bion®) (Friedrich et al. (1996) Plant J 10(1):61-70; Lawton et al. (1996) Plant J. 10:71-82). The expression of pathogenesis-related (PR) proteins, which are upregulated in the case of SAR, may also cause pathogen resistance in some cases.

In barley, the Mlo locus has been described for some time as a negative regulator of plant defense. The loss, or loss of function, of the Mlo gene causes an increased and, above all, race-unspecific resistance for example against a large number of mildew species (Büschges R et al. (1997) Cell 88:695-705; Jorgensen JH (1977) Euphytica 26:55-62; Lyngkjaer MF et al. (1995) Plant Pathol 44:786-790). The Mlo gene has only recently been cloned (Büschges R et al. (1997) Cell 88:695-705; WO 98/04586; Schulze-Lefert P, Vogel J (2000) Trends Plant Sci. 5:343-348). Various methods for obtaining pathogen resistance using these genes have been described (WO 98/04586; WO 00/01722; WO 99/47552). It is unclear whether an Mlo-based approach can also be performed successfully in dicotyledonous plants.

Phytopathogenic fungal species generally live as saprophytes or parasites. The latter depend - at least during certain phases of their lifecycle - on a supply of active substances (for example a supply of vitamins, carbohydrates and the like), as it can only be provided in this form by live plant cells. The expert classifies parasitic fungi as necrotrophic, hemibiotrophic and biotrophic. In the case of necrotrophic fungal parasites, the infection results in destruction of the tissue and thus in the death of the plant. In most cases, these fungi are only facultative

parasites; they are just as capable of saprophytic multiplication in dead or dying plant material.

Biotrophic fungal parasites are characterized in that parasite and host cohabit, at least over prolonged periods. While the fungus withdraws nutrients from the host, it does not kill it. Most biotrophic fungi are obligate parasites. Hemibiotrophic fungi live temporarily as biotrophs and kill the host at a later point in time, i.e. they enter a necrotrophic phase.

A further, large group of biotrophic plant pathogens of enormous agro-economical importance are nematodes. Phytopathogenic nematodes feed on the outermost parts of plant tissue (ectoparasites) or, after penetration into the plant, in cell layers further in (endoparasites). Two groups of endoparasitic root nematodes are distinguished according to their lifestyle and nutrition: cyst nematodes (Heterodera and Globodera species) and root-knot nematodes (Meloidogyne species). Both groups are obligate biotrophic parasites which induce the development of specific feeder cells in the roots. These feeder cells are plant cells whose metabolism has been modified by the nematodes in such a way that they specifically serve the supply of nutrients to the developing nematodes. The development of endoparasitic root nematodes depends totally on these feeder cells (for a review, see Sijmons et al. (1994) Ann. Rev. Phytopathol. 32: 235-259). Cyst nematodes (Heterodera and Globodera species) remain at the parasitization site in the root (sessile endoparasites) and convert the surrounding cells by protoplast fusion into syncytia while dissolving some of the cell walls. The nematodes feed off these feeder cells, which are formed in the root's central cylinder, during which process the nematodes swell greatly in size. Root-knot nematodes (Meloidogyne species) likewise remain at the parasitization site, once chosen, and bring about the development of feeder cells which, in contrast to the cyst nematodes, consist of several, multinucleate giant cells which develop as a result of synchronous divisions of the nucleus without cell wall formation (Fenoll and Del Campo (1998) Physiol. Mol. Biol. Plants 4:9-18). The development of the feeder cell systems is induced by signal molecules in the nematodes' saliva. It is known that a series of plant genes change their expression profile greatly during these differentiation processes. Promoters which are induced specifically in the feeder cell system (syncytia) are described in the literature. Those which may be mentioned by way of example are the tobacco Δ0.3 TobRB7 promoter (Opperman et al. (1994) Science 263:221-223), the tomato Lemmi9 promoter (Ecobar et al. (1999) Mol Plant Microbe

Interact 12: 440-449) and the geminivirus V sense promoters (WO 00/01832).

WO 94/10320 describes DNA constructs for the expression of genes which act as inhibitors of endogenous plant genes (for example ATP synthase, cytochrome C, pyruvate kinase) under the control of nematode-induced promoters in den syncytia.

Despite several advances in some fields of plant biotechnology, success in achieving a pathogen resistance in plants has been very limited and as yet only sufficiently documented in the case of viruses. Yield losses in particular as a result of fungal and nematode attack are a serious problem; then as now, they require an intensive application of fungicides and nematicides. However, the problems which this entails have still not been tackled sufficiently.

The present invention is based on the object of providing novel methods for pathogen defense, in plants, which bring about an effective defense of as broad a pathogen spectrum as possible, preferably a spectrum of fungi and nematodes, in as many different plant species as possible, preferably in agricultural crop plants. This object is achieved by the method according to the invention.

A first subject matter of the invention comprises a method for generating or increasing the resistance to at least one pathogen in plant organisms, which comprises the following process steps

a) transgenic expression of a protein with sucrose isomerase activity in a plant organism or a tissue, organ, part or cell thereof, and

b) selection of those plant organisms in which, as opposed or as compared to the original plant, the resistance to at least one pathogen exists or is increased.

In principle, the method according to the invention can be applied to all plant organisms which produce sucrose. This includes all higher plants. Surprisingly, it was observed that the growth of the fungus Alternaria is significantly inhibited on potato disks of transgenic potato plants in whose tubers sucrose is converted into palatinose owing to the recombinant expression of a sucrose isomerase.

It can also be observed that the recombinant expression of the sucrose isomerase also brings about a resistance to nematodes. In particular syncytia-specific expression of the sucrose isomerase sequence, caused by endoparasitic root nematodes, brings about a marked reduction of the nematode infestation.

Since a large number of pathogens, in particular fungi and nematodes, are not capable of metabolizing palatinose, overcoming the resistance by simple mutation in the pathogens is hardly possible since acquiring a novel enzyme activity would be required.

Within the context of the present invention, "protein with sucrose isomerase activity" refers to a protein whose "essential property" is the catalysis of the isomerization of sucrose to other disaccharides, the α1->β2-glycosidic bond between glucose and fructose in the sucrose being converted into a different glycosidic bond between two monosaccharide units, in particular into an α1->α6 bond and/or an α1->α1 bond.

A sucrose isomerase activity can be measured indirectly via analyzing the resulting carbohydrates (for example palatinose content) in the manner with which the skilled worker is familiar, for example by analyzing ethanolic extracts of suitable biological material (for example material from a transgenic plant or a microorganism). Said extracts can be analyzed for example by HPLC and the sugars can be identified with reference to corresponding standards. An analytical method is described for example in WO 01/59136. Thus, to detect sucrose isomerase activity in plant extracts, leaf disks with a diameter of approximately 0.8 cm are extracted for 2 hours at 70°C with 100 µl of 80% ethanol and 10 mM HEPES buffer (pH 7.5). An aliquot of these extracts can be analyzed using an HPLC system, for example from Dionex, which can be equipped with a PA-1 (4 x 250 mm) column and a pulsed electrochemical detector. Prior to injection, the samples can be spun down for 2 minutes at 13 000 rpm. The sugars can subsequently be eluted after 4 minutes at 150 mM NaOH and a flow rate of 1 ml/min, using a 10-minute gradient from 0 to 1 M sodium acetate. The sugars can be identified and determined quantitatively with the corresponding standards from Sigma.

A protein with sucrose isomerase activity is especially preferably understood as a protein which is capable of isomerizing sucrose into palatinose and/or trehalulose, as essential property. The palatinose and trehalulose amounts to at least 2%, preferably at least 20%, especially preferably at least 50% and most prefer-

ably at least 60% of the total disaccharides which are formed by the isomerization of sucrose.

The nucleic acid sequence encoding a protein with sucrose isomerase activity can be isolated from natural sources or synthesized by traditional methods.

Examples of organisms whose cells comprise proteins with sucrose isomerase activity and the nucleic acid sequences encoding therefor are, in particular, microorganisms of the genera Protaminobacter, Erwinia, Serratia, Leuconostoc, Pseudomonas, Agrobacterium, Klebsiella and Enterobacter. The following examples of such microorganisms may be mentioned in particular in this context: Protaminobacter rubrum (CBS 547, 77), Erwinia rhapontici (NCPPB 1578), Serratia plymuthica (ATCC 15928), Serratia marcescens (NCIB 8285), Leuconostoc mesenteroides NRRL B-52 If (ATCC 1083 0a). Pseudomonas mesoacidophila MX-45 (FERM 11808 or FERM BP 3619), Agrobacterium radiobacter MX-232 (FERM 12397 or FERM BP 3620), Klebsiella subspecies and Enterobacter species.

In a preferred embodiment, the nucleic acid sequence encoding a protein with a sucrose isomerase activity comprises nucleic acid sequences which encode proteins with sucrose isomerase activity, the nucleic acids being selected from the group consisting of

i) nucleic acid sequences encoding a protein as shown in SEQ ID NO: 2, 6, 8, 10, 12, 14, 16, 18 or 36 and

ii) nucleic acid sequences encoding a functional equivalent to a protein as shown in SEQ ID NO: 2, 6, 8, 10, 12, 14, 16, 18 or 36 and

iii) nucleic acid sequences encoding functionally equivalent fragments to a protein as shown in i) and ii).

Further nucleic acid sequences which encode proteins with sucrose isomerase activity are known in the art and are thus available to the skilled worker for the transfer to plant cells. Thus, for example, sequences from Protaminobacter rubrum, Erwinia rhapontici, Enterobacter species SZ 62 and Pseudomonas mesoacidophila MX-45 are described in WO 95/20047. The disclosure of this patent application is expressly referred to herewith, both with regard to the disclosed sequences themselves and with regard to the identification and characterization of these and further sucrose-isomerase-encoding sequences from other sources.

Further DNA sequences which encode sucrose isomerases can be found by the skilled worker in, inter alia, the gene databases using suitable search profiles and computer programs for screening for homologous sequences or for sequence alignments. In addition, the skilled worker can himself find, and employ for the purposes of the present invention, further sucrose-isomerase-encoding nucleic acid sequences from other organisms by means of conventional molecular-biological techniques. Thus, the skilled worker can for example derive suitable hybridization probes from the known sucrose isomerase sequences and employ them for screening cDNA libraries and/or genomic libraries of the desired organism in question from which a novel sucrose isomerase gene is to be isolated. In this context, the skilled worker can resort to customary hybridization, cloning and sequencing techniques (see, for example, Sambrook et al. (1989) Molecular Cloning: A Laboratory Manual, 2nd edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York). Likewise, the skilled worker is capable of using PCR to synthesize, with the aid of known sucrose isomerase DNA sequences, suitable - optionally degenerate - oligonucleotides as primers for cloning novel genes; such primers can be employed successfully.

Proteins with sucrose isomerase activity and the nucleic acid sequences encoding them can be isolated without difficulty by the skilled worker in the familiar manner from those organisms in which such activities were detected. Suitable methods are described (for example DE 44 14 185). Thus, a genetic library whose clones comprise genomic segments, of between 2 and 15 kb, of the donor organism can be obtained for example by partial digestion of genomic DNA of such an organism (preferably a microorganism) and introducing the resulting fragments into suitable E. coli vectors, followed by transformation. Among E. coli cells which harbor these plasmids, those whose colonies develop a red coloration are selected by plating on McConkey palatinose medium. The plasmid DNA present in these cells is transferred into an E. coli mutant which is not capable of growth on galactose as the single C source (for example ED 8654, Sambrook et al., supra, pages A9-A13). This transformed cell line is capable of identifying palatinose producers in the genetic library which has been generated as described above with DNA of the donor organism. To identify the desired palatinose-forming clones, individual cells from the genetic library are grown on minimal salt media supplemented with galactose and sucrose. Following replica-plating of the clonies onto plates with the same medium, the cells are killed by applying toluene vapor. Cells of the screening strain are subsequently applied above the colonies of the genetic library in the form of a lawn in soft minimal salt agar without

added C source, and incubated. Significant cell growth of the screening strain only develops at the location of cells of the genetic library which have produced palatinose. Testing the cells of the replica control reveals the isomerase content. The E. coli clones identified thus are not capable of growing on palatinose as the single C source in the medium, show no ability to cleave sucrose when the intact cells or cell extracts are tested, but will form palatinose when grown under these conditions and without addition of sucrose to the medium.

Alternatively, isomerase clones can also be identified using a PCR fragment. If plasmid DNA of the thus-identified E. coli clones is used as probes for hybridization on filters with immobilized DNA from the donor organism, gene regions which bear isomerase genes can be detected and made available in a targeted manner.

Functional equivalents of the proteins with sucrose isomerase activity disclosed within the present invention comprise preferably those from other organisms, for example from microorganisms, whose genomic sequence is known in its entirety or in part, such as, for example, from microorganisms of the genera Protaminobacter, Erwinia, Serratia, Leuconostoc, Pseudomonas, Agrobacterium, Klebsiella and Enterobacter. These can be identified for example by database search in sequence databases such as GenBank or by screening genetic libraries or cDNA libraries - for example using the sequence as shown in SEQ ID NO: 1 or a part thereof as search sequence or probe. Mutations comprise substitutions, additions, deletions, inversion or insertions of one or more amino acid residues.

If desired, the skilled worker can thus additionally resort to routine techniques in order to introduce various types of mutations into the sucrose-isomerase-encoding DNA sequence, which results in the synthesis of proteins whose biological properties may be modified. Thus, for example, it is possible specifically to prepare enzymes which are localized in specific compartments of the plant cell owing to addition of suitable signal sequences. Such sequences are described in the literature and known to the skilled worker (see, for example, Braun et al. (1992) EMBO J 11:3219-3227; Wolter F et al. (1988) Proc Natl Acad Sci USA 85:846-850; Sonnewald U et al. (1991) Plant J 1:95-106).

Also feasible is the introduction of point mutations at positions where for example a modification of the amino acid sequence affects, for example, enzyme activity or the regulation of the enzyme. In this manner it is possible, for example, to generate mu-

tants which are no longer subject to the regulatory mechanisms via allosteric regulation or covalent modification, mechanisms which normally prevail in the cell. Moreover, mutants with a modified substrate or product specificity can be generated. Moreover, mutants with a modified activity, temperature and/or pH profile can be generated.

The degeneracy of the genetic code allows the skilled worker, inter alia, to adapt the nucleotide sequence of the DNA sequence to the codon preference of the target plant, that is to say the plant or plant cell which is pathogen-resistant owing to the expression of the sucrose isomerase-nucleic acid sequence, thus optimizing expression.

In order to perform recombinant manipulation in prokaryotic cells, the recombinant nucleic acid molecules according to the invention, or parts thereof, can be introduced into plasmids which permit mutagenesis or sequence modification by recombination of DNA sequences. Base substitutions can be carried out, or natural or synthetic sequences added, with the aid of standard methods (see, for example, Sambrook et al. (1989), vide supra). To link the DNA fragments to one another, adaptors or linkers may be added to the fragments where required. Moreover, suitable restriction cleavage sites can be provided, or excess DNA or restriction cleavage sites eliminated, by means of enzymatic and other manipulations. Where insertions, deletions or substitutions are suitable, in-vitro mutagenesis, primer repair, restriction or ligation may be used. Analytical methods which are generally carried out are sequence analysis, restriction analysis and further biochemical/molecular-biological methods.

Said functional equivalents preferably have at least 40%, particularly preferably at least 50%, particularly preferably at least 70%, most preferably at least 90%, homology with one of the polypeptide sequences with the SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 or 36. The homology extends over at least 30 amino acids, preferably at least 60 amino acids, especially preferably at least 90 amino acids, most preferably over the entire length of one of the polypeptides as shown in SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 or 36.

Homology between two polypeptides is understood as meaning the identity of the amino acid sequence over in each case the entire sequence length which is calculated by comparison with the aid of the program algorithm GAP (Wisconsin Package Version 10.0,

University of Wisconsin, Genetics Computer Group (GCG), Madison, USA), setting the following parameters:

Gap weight: 8	Length weight: 2
Average match: 2,912	Average mismatch:-2,003

For example a sequence which has at least 80% homology with sequence SEQ ID NO: 2 at the protein level is understood as meaning a sequence which, upon comparison with the sequence SEQ ID NO: 2 by the above program algorithm with the above parameter set, has at least 80% homology.

The term functional equivalents comprises also those proteins which are encoded by nucleic acid sequences which have at least 40%, especially preferably at least 50%, especially preferably at least 70%, most preferably at least 90% homology with one of the nucleic acid sequences with the SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21 or 35. The homology extends over at least 100 bases, preferably at least 200 bases, especially preferably at least 300 bases, most preferably over the entire length of one of the sequences as shown in SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21 or 35.

Homology between two nucleic acid sequences is understood as meaning the identity of the two nucleic acid sequences over in each case the entire sequence length which is calculated by comparison with the aid of the program algorithm GAP (Wisconsin Package Version 10.0, University of Wisconsin, Genetics Computer Group (GCG), Madison, USA; Altschul et al. (1997) Nucleic Acids Res. 25:3389 et seq.), setting the following parameters:

Gap weight: 50	Length weight: 3
Average match: 10	Average mismatch:0

For example a sequence which has at least 80% homology with sequence SEQ ID NO: 1 at the nucleic acid level is understood as meaning a sequence which, upon comparison with the sequence SEQ ID NO: 1 by the above program algorithm with the above parameter set, has at least 80% homology.

The term functional equivalents also comprises those proteins which are encoded by nucleic acid sequences which hybridize under standard conditions with one of the nucleic acid sequences described by SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21 or 35, the nucleic acid sequence complementary thereto or parts of

the above, and which have the essential properties of a sucrose isomerase.

"Standard hybridization conditions" is to be understood in the broad sense and means stringent or else less stringent hybridization conditions. Such hybridization conditions are described, inter alia, by Sambrook J, Fritsch EF, Maniatis T et al., in Molecular Cloning (A Laboratory Manual), 2nd Edition, Cold Spring Harbor Laboratory Press, 1989, pages 9.31-9.57 or in Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6.

For example, the conditions during the wash step can be selected from the range of conditions delimited by low-stringency conditions (approximately 2X SSC at 50°C) and high-stringency conditions (approximately 0.2X SSC at 50°C, preferably at 65°C) (20X SSC: 0.3M sodium citrate, 3M NaCl, pH 7.0). In addition, the temperature during the wash step can be raised from low-stringency conditions at room temperature, approximately 22°C, to higher-stringency conditions at approximately 65°C. Both of the parameters salt concentration and temperature can be varied simultaneously, or else one of the two parameters can be kept constant while only the other is varied. Denaturants, for example formamide or SDS, may also be employed during the hybridization. In the presence of 50% formamide, hybridization is preferably effected at 42°C. Some examples of conditions for hybridization and wash step are shown hereinbelow:

(1) Hybridization conditions can be selected, for example, from the following conditions:

a) 4X SSC at 65°C,
b) 6X SSC at 45°C,
c) 6X SSC, 100 µg/ml denatured fragmented fish sperm DNA at 68°C,
f) 50% formamide, 4X SSC at 42°C,
g) 2X or 4X SSC at 50°C (low-stringency condition),
h) 30 to 40% formamide, 2X or 4X SSC at 42°C (low-stringency condition).

(2) Wash steps can be selected, for example, from the following conditions:

a) 0.015 M NaCl/0.0015 M sodium citrate/0.1% SDS at 50°C.
b) 0.1X SSC at 65°C.
c) 0.1X SSC, 0.5% SDS at 68°C.
d) 0.1X SSC, 0.5% SDS, 50% formamide at 42°C.

e) 0.2X SSC, 0.1% SDS at 42°C.

f) 2X SSC at 65°C (low-stringency condition).

In a preferred embodiment, the nucleic acid sequence encoding a protein with a sucrose isomerase activity comprises nucleic acid sequences which encode proteins with sucrose isomerase activity, the nucleic acids being selected from the group consisting of

a) nucleic acid sequences encoding an amino acid sequence as shown in SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 or 36, and

b) nucleic acid sequences encoding proteins with at least 40% homology with the sequence as shown in SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 or 36, and

c) nucleic acid sequences as shown in SEQ ID No: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21 or 35, and

d) nucleic acid sequences which are degenerated to a nucleic acid sequence of c), and

e) nucleic acid sequences with at least 40% homology with a nucleic acid sequence as shown in SEQ ID No: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21 or 35, and

f) nucleic acid sequences which hybridize with a complementary strand of the nucleic acid sequence as shown in SEQ ID No: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21 or 35,

and functionally equivalent fragments of the above.

With regard to a protein with sucrose isomerase activity or a nucleic acid sequence encoding such a protein, functionally equivalent fragments refers to all those polypeptides, or nucleic acid sequences encoding those polypeptides which, in comparison with their starting sequence, are truncated at the 5′ and/or 3′ end and/or have one or more deletions, but which still retain a sucrose isomerase activity, or still encode a protein with such an activity. One possibility in this context is the generation of deletion mutants where a progressive deletion from the 5′ or the 3′ end of the coding DNA sequence results in the synthesis of suitably truncated proteins.

As mentioned above, the coding sequences for sucrose isomerases can also be complemented by signal sequences which ensure that the gene product, i.e. predominantly the protein with sucrose isomerase activity, is transported into a particular compartment.

In a preferred embodiment of the invention, signal sequences ensure that the sucrose isomerase is transported into the cell wall or the apoplast of the transformed plant cells, i.e. the transformed plants express a chimeric sucrose isomerase, which comprises a signal peptide for the transport into the endoplasmic reticulum. Suitable signal sequences which ensure the uptake into the endoplasmic reticulum can be found by the skilled worker in the specialist literature. Especially suitable is for example the sequence which encodes the signal peptide of the potato proteinase inhibitor II gene (Keil et al. (1996) Nucl Acids Res 14:5641-5650; Genbank Accession No. X04118). Other suitable signal sequences ensure for example the uptake of sucrose isomerase into the vacuole. An example which may be mentioned is the signal peptide of the potato patatin gene (Sonnewald U et al. (1991) Plant J 1(1):95-106).

"Pathogen resistance" denotes the reduction or weakening of disease symptoms of a plant following infection by a pathogen. The symptoms can be manifold, but preferably comprise those which directly or indirectly have an adverse effect on the quality of the plant, the quantity of the yield, the suitability for use as feeding stuff or foodstuff, or else which make sowing, planting, harvesting or processing of the crop difficult.

"Conferring", "existing", "generating" or "increasing" a pathogen resistance means that the defense mechanisms of a specific plant species or variety is increasingly resistant to one or more pathogens due to the use of the method according to the invention in comparison with the wild type of the plant ("original plant"), to which the method according to the invention has not been applied, under otherwise identical conditions (such as, for example, climatic conditions, growing conditions, pathogen species and the like). The increased resistance manifests itself preferably in a reduced manifestation of the disease symptoms, disease symptoms comprising - in addition to the abovementioned adverse effects - for example also the penetration efficiency of a pathogen into the plant or plant cells or the proliferation efficiency in or on the same. In this context, the disease symptoms are preferably reduced by at least 10% or at least 20%, especially preferably by at least 40% or 60%, very especially

preferably by at least 70% or 80% and most preferably by at least 90% or 95%.

"Selection" with regard to plants in which - as opposed or as compared to the original plant - resistance to at least one pathogen exists or is increased means all those methods which are suitable for recognizing an existing or increased resistance to pathogens. These may be symptoms of pathogen infection (for example the development of haustoria in the case of fungal infection), but may also comprise the above-described symptoms which relate to the quality of the plant, the quantity of the yield, the suitability for use as feeding stuff or foodstuff and the like.

"Pathogen" within the scope of the invention means by way of example but not by limitation fungi, fungus-like pathogens (such as, for example, Chromista; e.g. Oomycetes) and animal pests such as, for example, nematodes. Especially preferred are nematodes and fungi. However, it can be assumed that the expression of a sucrose isomerase protein also brings about resistance to other pathogens.

The following pathogens may be mentioned by way of example but not by limitation:

1. Fungal pathogens and fungus-like pathogens:

   Fungal pathogens and fungus-like pathogens (such as, for example, Chromista) comprise biotrophic, hemibiotrophic and necrotrophic fungi and are preferably from the group comprising Plasmodiophoramycota, Oomycota, Ascomycota, Chytridiomycetes, Zygomycetes, Basidiomycota and Deuteromycetes (Fungi imperfecti). The pathogens mentioned in Tables 1 and 2 and the diseases with which they are associated may be mentioned by way of example but not by limitation.

Table 1: Fungal plant diseases

Disease	Pathogen
Leaf rust	Puccinia recondita
Yellow rust	P. striiformis
Powdery mildew	Erysiphe graminis
Glume blotch	Septoria nodorum
Leaf blotch	Septoria tritici
Ear fusarioses	Fusarium spp.

Disease	Pathogen
Eyespot	Pseudocercosporella herpotrichoides
Smut	Ustilago spp.
Bunt	Tilletia caries
Take-all	Gaeumannomyces graminis
Anthrocnose leaf blight Anthracnose stalk rot	Colletotrichum graminicola (teleomorph: Glomerella graminicola Politis); Glomerella tucumanensis (anamorph: Glomerella falcatum Went)
Aspergillus ear and kernel rot	Aspergillus flavus
Banded leaf and sheath spot	Rhizoctonia solani Kuhn = Rhizoctonia microsclerotia J. Matz (telomorph: Thanatephorus cucumeris)
Black bundle disease	Acremonium strictum W. Gams = Cephalosporium acremonium Auct. non Corda
Black kernel rot	Lasiodiplodia theobromae = Botryodiplodia theobromae
Borde blanco	Marasmiellus sp.
Brown spot (black spot, stalk rot)	Physoderma maydis
Cephalosporium kernel rot	Acremonium strictum = Cephalosporium acremonium
Charcoal rot	Macrophomina phaseolina
Corticium ear rot	Thanatephorus cucumeris = Corticium sasakii
Curvularia leaf spot	Curvularia clavata, C. eragrostidis, = C. maculans (teleomorph: Cochliobolus eragrostidis), Curvularia inaequalis, C. intermedia (teleomorph: Cochliobolus intermedius), Curvularia lunata (teleomorph: Cochliobolus lunatus), Curvularia pallescens (teleomorph: Cochliobolus pallescens), Curvularia senegalensis, C. tuberculata (teleomorph: Cochliobolus tuberculatus)
Didymella leaf spot	Didymella exitalis
Diplodia ear rot and stalk rot	Diplodia frumenti (teleomorph: Botryosphaeria festucae)
Diplodia ear rot, stalk rot, seed rot and seedling blight	Diplodia maydis = Stenocarpella maydis
Diplodia leaf spot or streak	Stenocarpella macrospora = Diplodialeaf macrospora

Table 2: Downy mildew (Oomycetes)

Disease	Pathogen
Brown stripe downy mildew	Sclerophthora rayssiae var. zeae
Crazy top downy mildew	Sclerophthora macrospora = Sclerospora macrospora
Green ear downy mildew (graminicola downy mildew)	Sclerospora graminicola
Java downy mildew	Peronosclerospora maydis = Sclerospora maydis
Philippine downy mildew	Peronosclerospora philippinensis = Sclerospora philippinensis
Sorghum downy mildew	Peronosclerospora sorghi = Sclerospora sorghi
Spontaneum downy mildew	Peronosclerospora spontanea = Sclerospora spontanea
Sugarcane downy mildew	Peronosclerospora sacchari = Sclerospora sacchari
Dry ear rot (cob, kernel and stalk rot)	Nigrospora oryzae (teleomorph: Khuskia oryzae)
Ear rots, minor	Alternaria alternata = A. tenuis, Aspergillus glaucus, A. niger, Aspergillus spp., Botrytis cinerea (teleomorph: Botryotinia fuckeliana), Cunninghamella sp., Curvularia pallescens, Doratomyces stemonitis = Cephalotrichum stemonitis, Fusarium culmorum, Gonatobotrys simplex, Pithomyces maydicus, Rhizopus microsporus Tiegh., R. stolonifer = R. nigricans, Scopulariopsis brumptii
Ergot (horse's tooth)	Claviceps gigantea (anamorph: Sphacelia sp.)
Eyespot	Aureobasidium zeae = Kabatiella zeae
Fusarium ear and stalk rot	Fusarium subglutinans = F. moniliforme var.subglutinans
Fusarium kernel, root and stalk rot, seed rot and seedling blight	Fusarium moniliforme (teleomorph: Gibberella fujikuroi)
Fusarium stalk rot, seedling root rot	Fusarium avenaceum (teleomorph: Gibberella avenacea)
Gibberella ear and stalk rot	Gibberella zeae (anamorph: Fusarium graminearum)
Gray ear rot	Botryosphaeria zeae = Physalospora zeae (anamorph: Macrophoma zeae)
Gray leaf spot (Cercospora leaf spot)	Cercospora sorghi = C. sorghi var. maydis, C. zeae-maydis

Disease	Pathogen
Helminthosporium root rot	Exserohilum pedicellatum = Helminthosporium pedicellatum (teleomorph: Setosphaeria pedicellata)
Hormodendrum ear rot (Cladosporium rot)	Cladosporium cladosporioides = Hormodendrum cladosporioides, C. herbarum (teleomorph: Mycosphaerella tassiana)
Hyalothyridium leaf spot	Hyalothyridium maydis
Late wilt	Cephalosporium maydis
Leaf spots, minor	Alternaria alternata, Ascochyta maydis, A. tritici, A. zeicola, Bipolaris victoriae = Helminthosporium victoriae (teleomorph: Cochliobolus victoriae), C. sativus (anamorph: Bipolaris sorokiniana = H. sorokinianum = H. sativum), Epicoccum nigrum, Exserohilum prolatum = Drechslera prolata (teleomorph: Setosphaeria prolata) Graphium penicillioides, Leptosphaeria maydis, Leptothyrium zeae, Ophiosphaerella herpotricha, (anamorph: Scolecosporiella sp.), Paraphaeosphaeria michotii, Phoma sp., Septoria zeae, S. zeicola, S. zeina
Northern corn leaf blight (white blast, crown stalk rot, stripe)	Setosphaeria turcica (anarnorph: Exserohilum turcicum = Helminthosporium turcicum)
Northern corn leaf spot Helminthosporium ear rot (race 1)	Cochliobolus carbonum (anamorph: Bipolaris zeicola = Helminthosporium carbonum)
Penicillium ear rot (blue eye, blue mold)	Penicillium spp., P. chrysogenum, P. expansum, P. oxalicum
Phaeocytostroma stalk rot and root rot	Phaeocytostroma ambiguum, = Phaeocytosporella zeae
Phaeosphaeria leaf spot	Phaeosphaeria maydis = Sphaerulina maydis
Physalospora ear rot (Botryosphaeria ear rot)	Botryosphaeria festucae = Physalospora zeicola (anamorph: Diplodia frumenti)
Purple leaf sheath	Hemiparasitic bacteria and fungi
Pyrenochaeta stalk rot and root rot	Phoma terrestris = Pyrenochaeta terrestris
Pythium root rot	Pythium spp., P. arrhenomanes, P. graminicola
Pythium stalk rot	Pythium aphanidermatum = P. butleri L.
Red kernel disease (ear mold, leaf and seed rot)	Epicoccum nigrum

Disease	Pathogen
Rhizoctonia ear rot (sclerotial rot)	Rhizoctonia zeae (teleomorph: Waitea circinata)
Rhizoctonia root rot and stalk rot	Rhizoctonia solani, Rhizoctonia zeae
Root rots, minor	Alternaria alternata, Cercospora sorghi, Dictochaeta fertilis, Fusarium acuminatum (teleomorph: Gibberella acuminata), F. equiseti (teleomorph: G. intricans), F. oxysporum, F. pallidoroseum, F. poae, F. roseum, G. cyanogena, (anamorph: F. sulphureum), Microdochium bolleyi, Mucor sp., Periconia circinata, Phytophthora cactorum, P. drechsleri, P. nicotianae var. parasitica, Rhizopus arrhizus
Rostratum leaf spot (Helminthosporium leaf disease, ear and stalk rot)	Setosphaeria rostrata, (anamorph: Exserohilum rostratum = He/minthosporium rostratum)
Rust, common corn	Puccinia sorghi
Rust, southern corn	Puccinia polysora
Rust, tropical corn	Physopella pallescens, P. zeae = Angiopsora zeae
Sclerotium ear rot (southern blight)	Sclerotium rolfsii Sacc. (teleomorph: Athelia rolfsii)
Seed rot-seedling blight	Bipolaris sorokiniana, B. zeicola = Helminthosporium carbonum, Diplodia maydis, Exserohilum pedicillatum, Exserohilum turcicum = Helminthosporium turcicum, Fusarium avenaceum, F. culmorum, F. moniliforme, Gibberella zeae (anamorph: F. graminearum), Macrophomina phaseolina, Penicillium spp., Phomopsis sp., Pythium spp., Rhizoctonia solani, R. zeae, Sclerotium rolfsii, Spicaria sp.
Selenophoma leaf spot	Selenophoma sp.
Sheath rot	Gaeumannomyces graminis
Shuck rot	Myrothecium gramineum
Silage mold	Monascus purpureus, M ruber
Smut, common	Ustilago zeae = U. maydis
Smut, false	Ustilaginoidea virens
Smut, head	Sphacelotheca reiliana = Sporisorium holcisorghi
Southern corn leaf blight and stalk rot	Cochliobolus heterostrophus (anamorph: Bipolaris maydis = Helminthosporium maydis)

Disease	Pathogen
Southern leaf spot	Stenocarpella macrospora = Diplodia macrospora
Stalk rots, minor	Cercospora sorghi, Fusarium episphaeria, F. merismoides, F. oxysporum Schlechtend, F. poae, F. roseum, F. solani (teleomorph: Nectria haematococca), F. tricinctum, Mariannaea elegans, Mucor sp., Rhopographus zeae, Spicaria sp.
Storage rots	Aspergillus spp., Penicillium spp. and other fungi
Tar spot	Phyllachora maydis
Trichoderma ear rot and root rot	Trichoderma viride = T. lignorum teleomorph: Hypocrea sp.
White ear rot, root and stalk rot	Stenocarpella maydis = Diplodia zeae
Yellow leaf blight	Ascochyta ischaemi, Phyllosticta maydis (teleomorph: Mycosphaerella zeae-maydis)
Zonate leaf spot	Gloeocercospora sorghi

The following are especially preferred

- Plasmodiophoromycota such as Plasmodiophora brassicae (clubroot of crucifers), Spongospora subterranea (powdery scab of potato tubers), Polymyxa graminis (root disease of cereals and grasses),

- Oomycota such as Bremia lactucae (downy mildew of lettuce), Peronospora (downy mildew) in snapdragon (P. antirrhini), onion (P. destructor), spinach (P. effusa), soybean (P. manchurica), tobacco ("blue mold"; P. tabacina), alfalfa and clover (P. trifolium), Pseudoperonospora humuli (downy mildew of hops), Plasmopara (downy mildew in grapevines) (P. viticola) and sunflower (P. halstedii), Sclerophtohra macrospora (downy mildew in cereals and grasses), Pythium (seed rot, seedling damping-off, and root rot and all types of plants, for example damping-off of Beta beet caused by P. debaryanum), Phytophthora infestans (blight in potato, brown rot in tomato and the like), Albugo spec. (white rust on cruciferous plants).

- Ascomycota such as Microdochium nivale (snow mold of rye and wheat), Fusarium graminearum, Fusarium culmorum (partial ear sterility mainly in wheat), Fusarium oxysporum (Fusarium wilt of tomato), Blumeria graminis (powdery mildew of barley

(f.sp. hordei) and wheat (f.sp. tritici)), Erysiphe pisi (powdery mildew of pea), Nectria galligena (Nectria canker of fruit trees), Uncinula necator (powdery mildew of grapevine), Pseudopeziza tracheiphila (red fire disease of grapevine), Claviceps purpurea (ergot on, for example, rye and grasses), Gaeumannomyces graminis (take-all on wheat, rye and other grasses), Magnaporthe grisea (rice blast disease), Pyrenophora graminea (leaf stripe of barley), Pyrenophora teres (net blotch of barley), Pyrenophora tritici-repentis (leaf blight of wheat), Venturia inaequalis (apple scab), Sclerotinia sclerotium (stalk break, stem rot), Pseudopeziza medicaginis (leaf spot of alfalfa, white and red clover).

- Basidiomycetes such as Typhula incarnata (typhula blight on barley, rye, wheat), Ustilago maydis (blister smut on maize), Ustilago nuda (loose smut on barley), Ustilago tritici (loose smut on wheat, spelt), Ustilago avenae (loose smut on oats), Rhizoctonia solani (rhizoctonia root rot of potato), Sphacelotheca spp. (head smut of sorghum), Melampsora lini (rust of flax), Puccinia graminis (stem rust of wheat, barley, rye, oats), Puccinia recondita (leaf rust on wheat), Puccinia dispersa (brown rust on rye), Puccinia hordei (leaf rust of barley), Puccinia coronata (crown rust of oats), Puccinia striiformis (yellow rust of wheat, barley, rye and a large number of grasses), Uromyces appendiculatus (brown rust of bean), Sclerotium rolfsii (root and stem rots of many plants).

- Deuteromycetes (Fungi imperfecti) such as Septoria nodorum (glume blotch) of wheat (Septoria tritici), Pseudocercosporella herpotrichoides (eyespot of wheat, barley, rye), Rynchosporium secalis (leaf spot on rye and barley), Alternaria solani (early blight of potato, tomato), Phoma betae (blackleg on Beta beet), Cercospora beticola (leaf spot on Beta beet), Alternaria brassicae (black spot on oilseed rape, cabbage and other crucifers), Verticillium dahliae (verticillium wilt), Colletotrichum lindemuthianum (bean anthracnose), Phoma lingam (blackleg of cabbage and oilseed rape), Botrytis cinerea (grey mold of grapevine, strawberry, tomato, hops and the like).

Most preferred are Phytophthora infestans (potato blight, brown rot in tomato and the like), Microdochium nivale (previously Fusarium nivale; snow mold of rye and wheat), Fusarium graminearum, Fusarium culmorum (partial ear sterility of wheat), Fusarium oxysporum (Fusarium wilt of tomato), Blumeria graminis (powdery mildew of barley (f. sp. hordei) and wheat (f. sp.

tritici)), Magnaporthe grisea (rice blast disease), Sclerotinia sclerotium (stalk break, stem rot), Septoria nodorum and Septoria tritici (glume blotch of wheat), Alternaria brassicae (black spot of oilseed rape, cabbage and other crucifers), Phoma lingam (blackleg of cabbage and oilseed rape).

2. Animal pests

Among the plant-injurious nematodes which are preferred for the control within the scope of the present invention, the following groups may be mentioned by way of example, but not by limitation:

a) active, migratory root nematodes (for example Pratylenchus, Xiphinema and Longidorus species).

Migratory nematodes are not bound to one parasitization site, but can change the latter. They can migrate from one root to another, from one plant to another and in some cases also within the plant tissue. Their importance as pests has long been underrated. Nowadays, they are among the extremely dangerous plant-injurious nematodes. Many types of growth damage (including what is known as "cell sickness") and premature yellowing of the crop plants have been attributed to such root pests. Pratylenchus species in particular are also known in the cultivation of ornamentals as the cause of substantial root damage. Diseased roots can be recognized from sections of brown discoloration. Rot organisms subsequently enter the lesions caused, bring about rapid death of the tissue and pronounced rotting at these locations. Host plants are inter alia: various cereal species, potatoes, carrots, tomatoes, cucumbers, celery/celeriac and grapevine.

b) Root-gall-causing nematodes (for example Meloidogyne species)

The larvae of these species usually burrow into the roots close to the tip and, by virtue of exudations of their saliva glands, cause nuts (galls) in the surrounding plant tissue to form. They are sedentary in these galls, and return to the soil either actively or after decomposition of the galls. The adverse effect on the plant's metabolism, which is the result of the attack by these pests, can be seen from the more or less stunted growth and general failure of the plant to thrive. Root-knot eelworms are major pests in particular in greenhouses, but have also been identified in the open on carrots, celery/celeriac and parsley.

c) Nematodes which attack the floral primordia: (Anguina tritici)

The ear-cockle eelworm is a parasite which specializes in the floral primordia of wheat, which it converts into galls. Attack by this nematode can already be identified during the juvenile stage of the plant from the waviness or curliness of the leaves.

d) Cyst-forming root nematodes: (Globodera and Heterodera species)

The potato cyst nematode is Potato Enemy Number 1. Regarding its injuriousness, this species surpasses all the other Heterodera species; severe infestation can destroy up to 80% of the harvest. After infestation with cyst nematodes, the plant fails to thrive and no cause can be discerned externally. Only an examination of the roots reveals pinhead-sized brownish, yellow or whitish cysts are revealed. The female nematodes burrow into the root, which they burst by means of their abdomen, which is filled with eggs and thereby swelling. While the packed abdomen is still surrounded by the soil, the nematode's mouth spear is still attached to the root. The female dies, and its solidifying skin becomes a protective cover (cyst) for the eggs and larvae. The cysts together with their contents are very resilient and can persist for a long time. Under suitable environmental conditions, the larvae burrow into the open and infest fresh roots. The most important cyst nematodes are the potato cyst nematode, the beet cyst nematode, the cereal cyst nematode, the pea cyst nematode, the clover cyst nematode, the beet cyst eelworm, the hop cyst nematode, and the carrot cyst nematode (for the examination for potato cyst nematodes, see also: http://www.bfl.at/)

Preferred animal pests are in particular nematodes. Examples which may be mentioned, but not by limitation, are the pathogens mentioned in Table 3 and the diseases associated with them.

Table 3: Parasitic nematodes

Damage	Pathogenic nematode
Awl	Dolichodorus spp., D. heterocephalus
Bulb and stem nematode disease; Europe	Ditylenchus dipsaci
Burrowing	Radopholus similis
Cyst nematode disease	Heterodera avenae, H. zeae, Punctodera chalcoensis
Dagger	Xiphinema spp., X. americanum, X. mediterraneum
False root-knot	Nacobbus dorsalis
Lance, Columbia	Hoplolaimus columbus

Damage	Pathogenic nematode
Lance	Hoplolaimus spp., H. galeatus
Lesion	Pratylenchus spp., P. brachyurus, P. crenatus, P. hexincisus, P. neglectus, P. penetrans, P. scribneri, P. thornei, P. zeae
Needle	Longidorus spp., L. breviannulatus
Ring	Criconemella spp., C. ornata
Root-knot disease	Meloidogyne spp., M. chitwoodi, M. incognita, M. javanica
Spiral	Helicotylenchus spp.
Sting	Belonolaimus spp., B. longicaudatus
Stubby-root	Paratrichodorus spp., P. christiei, P. minor, Quinisulcius acutus, Trichodorus spp.
Stunt	Tylenchorhynchus dubius

Very especially preferred are Globodera rostochiensis and G. pallida (cyst eelworm on potato, tomato and other Solanaceae), Heterodera schachtii (beet eelworm on sugar and fodder beet, oilseed rape, cabbage and the like), Heterodera avenae (cereal cyst nematode on oat and other cereal species), Ditylenchus dipsaci (stem or bulb eelworm, stem eelworm of rye, oats, maize, clover, tobacco, beet), Anguina tritici (ear-cockle nematode, cockle disease of wheat (spelt, rye), Meloidogyne hapla (root-knot nematode of carrot, cucumber, lettuce, tomato, potato, sugar beet, lucerne).

It is preferred to obtain a resistance to the following examples of fungal pathogens in the individual plant species:

1. Barley: Puccinia graminis f.sp. hordei (barley stem rust), Blumeria (Erysiphe) graminis f.sp. hordei (barley powdery mildew).

2. Soybean: Phytophthora megasperma fsp.glycinea, Macrophomina phaseolina, Rhizoctonia solani, Sclerotinia sclerotiorum, Fusarium oxysporum, Diaporthe phaseolorum var. sojae (Phomopsis sojae), Diaporthe phaseolorum var. caulivora, Sclerotium rolfsii, Cercospora kikuchii, Cercospora sojina, Peronospora manshurica, Colletotrichum dematium (Colletotrichum truncatum), Corynespora cassiicola, Septoria glycines, Phyllosticta sojicola, Alternaria alternata, Microsphaera diffusa, Fusarium semitectum, Phialophora gregata, Glomerella glycines,

Phakopsora pachyrhizi, Pythium aphanidermatum, Pythium ultimum, Pythium debaryanum, Fusarium solani.

3. Canola: Albugo candida, Alternaria brassicae, Leptosphaeria maculans, Rhizoctonia solani, Sclerotinia sclerotiorum, Mycosphaerella brassiccola, Pythium ultimum, Peronospora parasitica, Fusarium roseum, Alternaria alternata.

4. Alfalfa: Clavibater michiganese subsp. insidiosum, Pythium ultimum, Pythium irregulare, Pythium splendens, Pythium debaryanum, Pythium aphanidermatum, Phytophthora megasperma, Peronospora trifoliorum, Phoma medicaginis var. medicaginis, Cercospora medicaginis, Pseudopeziza medicaginis, Leptotrochila medicaginis, Fusarium, Aphanomyces euteiches, Stemphylium herbarum, Stemphylium alfalfae.

5. Wheat: Urocystis agropyri, Alternaria alternata, Cladosporium herbarum, Fusarium graminearum, Fusarium avenaceum, Fusarium culmorum, Ustilago tritici, Ascochyta tritici, Cephalosporium gramineum, Collotetrichum graminicola, Erysiphe graminis f.sp. tritici, Puccinia graminis f.sp. tritici, Puccinia recondita f.sp. tritici, Puccinia striiformis, Pyrenophora tritici-repentis, Septoria nodorum, Septoria tritici, Septoria avenae, Pseudocercosporella herpotrichoides, Rhizoctonia solani, Rhizoctonia cerealis, Gaeumannomyces graminis var. tritici, Pythium aphanidermatum, Pythium arrhenomanes, Pythium ultimum, Bipolaris sorokiniana, Claviceps purpurea, Tilletia tritici, Tilletia laevis, Ustilago tritici, Tilletia indica, Rhizoctonia solani, Pythium arrhenomannes, Pythium gramicola, Pythium aphanidermatum, Puccinia graminis f.sp. tritici (wheat stem rust), Blumeria (Erysiphe) graminis f.sp. tritici (wheat powdery mildew).

6. Sunflower: Plasmophora halstedii, Sclerotinia sclerotiorum, Aster Yellows, Septoria helianthi, Phomopsis helianthi, Alternaria helianthi, Alternaria zinniae, Botrytis cinerea, Phoma macdonaldii, Macrophomina phaseolina, Erysiphe cichoracearum, Rhizopus oryzae, Rhizopus arrhizus, Rhizopus stolonifer, Puccinia helianthi, Verticillium dahliae, Cephalosporium acremonium, Phytophthora cryptogea, Albugo tragopogonis.

7. Maize: Fusarium moniliforme var. subglutinans, Fusarium moniliforme, Gibberella zeae (Fusarium graminearum), Stenocarpella maydi (Diplodia maydis), Pythium irregulare, Pythium debaryanum, Pythium graminicola, Pythium splendens, Pythium ultimum, Pythium aphanidermatum, Aspergillus flavus, Bipolaris maydis 0, T (Cochliobolus heterostrophus), Helminthosporium

carbonum I, II & III (Cochliobolus carbonum), Exserohilum turcicum I, II & III, Helminthosporium pedicellatum, Physoderma maydis, Phyllosticta maydis, Kabatiella maydis, Cercospora sorghi, Ustilago maydis, Puccinia sorghi, Puccinia polysora, Macrophomina phaseolina, Penicillium oxalicum, Nigrospora oryzae, Cladosporium herbarum, Curvularia lunata, Curvularia inaequalis, Curvularia pallescens, Trichoderma viride, Claviceps sorghi, Cornstunt spiroplasma, Diplodia macrospora, Sclerophthora macrospora, Peronosclerospora sorghi, Peronosclerospora philippinesis, Peronosclerospora maydis, Peronosclerospora sacchari, Spacelotheca reiliana, Physopella zeae, Cephalosporium maydis, Caphalosporium acremonium.

8. Sorghum: Exserohilum turcicum, Colletotrichum graminicola (Glomerella graminicola), Cercospora sorghi, Gloeocercospora sorghi, Ascochyta sorghina, Puccinia purpurea, Macrophomina phaseolina, Perconia circinata, Fusarium moniliform, Alternaria alternata, Bipolaris sorghicola, Helminthosporium sorghicola, Curvularia lunata, Phoma insidiosa, Ramulispora sorghi, Ramulispora sorghicola, Phyllachara sacchari, Sporisorium reilianum (Sphacelotheca reiliana), Sphacelotheca cruenta, Sporisorium sorghi, Claviceps sorghi, Rhizoctonia solani, Acremonium strictum, Sclerophthona macrospora, Peronosclerospora sorghi, Peronosclerospora philippinensis, Sclerospora graminicola, Fusarium graminearum, Fusarium oxysporum, Pythium arrhenomanes, Pythium graminicola.

It is preferred to obtain, in the individual plant species, a resistance to the following nematode pathogens which are mentioned by way of example:

Sugar beet	Sugar beet cyst nematode	Heterodera schachtii
Potato	Columbia root-knot nematode	Meloidogyne chitwoodi
	Golden Nematode	*Globodera rostochiensis*
	Northern root knot nematode	Meloidogyne hapla
	Potato rot nematode	Ditylenchus destructor
Soybean	Soybean cyst nematode; SCN	Heterodera glycines
Maize	Corn cyst nematode	Heterodera zeae
	Root-knot nematodes	Meloidogyne species: Meloidogyne arenaria Meloidogyne graminicola Meloidogyne chitwoodi Meloidogyne hapla Meloidogyne incognita Meloidogyne javanica

"Plant organism or cells derived therefrom" generally refers to any cell, tissue, part or propagation material (such as seeds or fruits) of an organism which is capable of photosynthesis. Included for the purposes of the invention are all genera and species of higher and lower plants of the Plant Kingdom. Preferred plants are annual, perennial, monocotyledonous and dicotyledonous plants. Included are the mature plant, seed, shoots and seedlings, and parts, propagation material (for example tubers, seeds or fruits) and cultures, for example cell or callus cultures, which are derived therefrom. Mature plants refers to plants at any developmental stage beyond the seedling stage. Seedling refers to a young, immature plant in an early developmental stage.

The term "plant" in the context of the invention refers to all genera and species of higher and lower plants of the Plant Kingdom. The term includes the mature plants, seed, shoots and seedlings and parts, propagation material, plant organs, tissue, protoplasts, callus and other cultures, for example cell cultures, which are derived therefrom, and any other type of plant cell grouping to give functional or structural units. Mature plants refers to plants at any developmental stage beyond that of the seedling. Seedling refers to a young immature plant at an early developmental stage.

"Plant" comprises all annual and perennial monocotyledonous and dicotyledonous plants and includes by way of example but not by limitation those of the genera Cucurbita, Rosa, Vitis, Juglans, Fragaria, Lotus, Medicago, Onobrychis, Trifolium, Trigonella, Vigna, Citrus, Linum, Geranium, Manihot, Daucus, Arabidopsis, Brassica, Raphanus, Sinapis, Atropa, Capsicum, Datura, Hyoscyamus, Lycopersicon, Nicotiana, Solanum, Petunia, Digitalis, Majorana, Cichorium, Helianthus, Lactuca, Bromus, Asparagus, Antirrhinum, Heterocallis, Nemesis, Pelargonium, Panicum, Pennisetum, Ranunculus, Senecio, Salpiglossis, Cucumis, Browallia, Glycine, Pisum, Phaseolus, Lolium, Oryza, Zea, Avena, Hordeum, Secale, Triticum, Sorghum, Picea and Populus.

Preferred plants are those from the following plant families: Amaranthaceae, Asteraceae, Brassicaceae, Carophyllaceae, Chenopodiaceae, Compositae, Cruciferae, Cucurbitaceae, Labiatae, Leguminosae, Papilionoideae, Liliaceae, Linaceae, Malvaceae, Rosaceae, Rubiaceae, Saxifragaceae, Scrophulariaceae, Solanaceae, Sterculiaceae, Tetragoniacea, Theaceae, Umbelliferae.

Preferred monocotyledonous plants are selected in particular from the monocotyledonous crop plants such as, for example, the family of the Gramineae such as rice, maize, wheat or other cereal spe-

cies such as barley, sorghum and millet, rye, triticale or oats, and sugar cane, and all grass species.

The invention is very especially preferably applied to dicotyledonous plant organisms. Preferred dicotyledonous plants are in particular selected among the dicotyledonous crop plants such as, for example,

- Asteraceae, such as sunflower, Tagetes or Calendula and others,

- Compositae, in particular the genus Lactuca, especially the species sativa (lettuce) and others,

- Cruciferae, especially the genus Brassica, very especially the species napus (oilseed rape), campestris (beet), oleracea cv Tastie (cabbage), oleracea cv Snowball Y (cauliflower) and oleracea cv Emperor (broccoli) and other cabbage species; and the genus Arabidopsis, very especially the species thaliana, and cress or canola and others,

- Cucurbitaceae, such as melon, pumpkin/squash or courgette and others,

- Leguminosae, especially the genus Glycine, very especially the species max (soybean) and alfalfa, pea, beans or peanut, and others

- Rubiaceae, preferably the subclass Lamiidae, such as, for example Coffea arabica or Coffea liberica (coffee bush) and others,

- Solanaceae, in particular the genus Lycopersicon, very especially the species esculentum (tomato), the genus Solanum, very especially the species tuberosum (potato) and melongena (aubergine), and the genus Capsicum, very especially the species annuum (bell pepper) and tobacco and others,

- Sterculiaceae, preferably the subclass Dilleniidae, such as, for example, Theobroma cacao (cacao tree) and others,

- Theaceae, preferably the subclass Dilleniidae, such as, for example, Camellia sinensis or Thea sinensis (tee shrub) and others,

- Umbelliferae, especially the genus Daucus (very especially the species carota (carrot)) and Apium (very especially the species graveolens dulce (celery)) and others,

and linseed, soybean, cotton, hemp, flax, cucumber, spinach, carrot, sugar beet and the various tree, nut and grapevine species, in particular banana and kiwi.

Also comprised are ornamental plants, useful trees, ornamental trees, flowers, cut flowers, shrubs or lawn. Those which must be mentioned by way of example, but not by limitation, are angiosperms, bryophytes such as, for example, Hepaticae (liverworts) and Musci (mosses); pteridophytes such as ferns, mare's tail, Lycopodiaceae; gymnosperms such as conifers, cycads, ginkgo and Gnetatae, the families of the Rosaceae such as rose, Ericaceae such as rhododendrons and azaleas, Euphorbiaceae such as poinsettias and croton, Caryophyllaceae such as carnations, Solanaceae such as petunias, Gesneriaceae such as African violet, Balsaminaceae such as touch-me-not, Orchidaceae such as orchids, Iridaceae such as gladioli, iris, freesia and crocus, Compositae such as calendula, Geraniaceae such as geraniums, Liliaceae such as dracaena, Moraceae such as ficus, Araceae such as philodendron and many others.

Most preferred are agricultural crop plants which naturally have a high sucrose content or whose roots, tubers or storage roots are of economic utility, such as, for example, potato, beet or sugar beet. Likewise preferred are tomato, banana, carrot, sugar cane, strawberry, pineapple, paw paw, soybean and cereal species such as oats, barley, wheat, rye, triticale, sorghum and millet, and maize. Most preferred are potato, beet, sugar beet and sugar cane.

Within the present invention, expression constructs for expressing proteins with sucrose isomerase activity in plants are employed. Such expression cassettes are described for example in WO 01/59136 and WO 01/59135, which are expressly referred to herewith.

In said expression constructs, a nucleic acid molecule encoding a protein with sucrose isomerase activity (for example described by SEQ ID NO: 2 or a functional equivalent thereof or a functional equivalent part of the above) is preferably operably linked to at least one genetic control element (for example a promoter) which ensures recombinant expression in a plant organism or a tissue, organ, part or cell thereof.

Operable linkage is to be understood as meaning, for example, the sequential arrangement of a promoter with the nucleic acid sequence to be expressed (for example the sequence as shown in SEQ ID NO: 1) and, if appropriate, further regulatory elements

such as, for example, a terminator in such a way that each of the regulatory elements can fulfill its function when the nucleic acid sequence is expressed recombinantly. To this end, direct linkage in the chemical sense is not necessarily required. Genetic control sequences such as, for example, enhancer sequences, can also exert their function on the target sequence from positions which are further away, or indeed from other DNA molecules. Preferred arrangements are those in which the nucleic acid sequence to be expressed recombinantly is positioned behind the sequence acting as promoter, so that the two sequences are linked covalently to each other.

Operable linkage, and an expression construct, can be generated by means of customary recombination and cloning techniques as are described, for example, in Maniatis T, Fritsch EF and Sambrook J (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor (NY), in Silhavy TJ, Berman ML and Enquist LW (1984) Experiments with Gene Fusions, Cold Spring Harbor Laboratory, Cold Spring Harbor (NY), in Ausubel FM et al. (1987) Current Protocols in Molecular Biology, Greene Publishing Assoc. and Wiley Interscience and in Gelvin et al. (1990) In: Plant Molecular Biology Manual. However, further sequences which, for example, act as a linker with specific cleavage sites for restriction enzymes, as att sequence for recombinases or as a signal peptide, may also be positioned between the two sequences. The insertion of sequences may also lead to the expression of fusion proteins. Preferably, the transgenic expression construct, consisting of a linkage of promoter and nucleic acid sequence to be expressed, can exist in a vector-integrated form and be inserted into a plant genome, for example by transformation.

However, an expression construct also denotes those constructions in which the nucleic acid sequence encoding the protein with sucrose isomerase activity (for example encoded by SEQ ID NO: 2 or a functional equivalent thereof or a functionally equivalent part of the above) is placed behind an endogenous plant promoter - for example by homologous recombination - in such a way that this promoter ensures the recombinant expression of said nucleic acid sequence.

The term plant-specific promoters is understood as meaning, in principle, any promoter which is capable of governing the expression of genes, in particular foreign genes, in plants or plant parts, plant cells, plant tissues, or plant cultures. Here, the promoter may be chosen in such a way that expression is constitutive or only in a specific tissue or organ, at a particular point in time of the plant development and/or at a point in time which

is determined by external factors, biotic or abiotic stimuli (induced gene expression). With regard to the plant to be transformed, the promoter may be homologous or heterologous. The following are preferred:

a) Constitutive promoters

"Constitutive" promoter is understood as meaning those promoters which ensure expression in a large number of, preferably all, tissues over a substantial period of plant development, preferably at all stages of plant development (Benfey et al.(1989) EMBO J 8:2195-2202). In particular a plant promoter or a promoter derived from a plant virus are preferably used. Particularly preferred is the promoter of the CaMV cauliflower mosaic virus 35S transcript (Franck et al. (1980) Cell 21:285-294; Odell et al. (1985) Nature 313:810-812; Shewmaker et al. (1985) Virology 140:281-288; Gardner et al. (1986) Plant Mol Biol 6:221-228) or the 19S CaMV promoter (US 5,352,605; WO 84/02913; Benfey et al. (1989) EMBO J 8:2195-2202). Another suitable constitutive promoter is the leguminB promoter (GenBank Acc. No. X03677), the Agrobacterium nopaline synthase promoter, the TR dual promoter, the Agrobacterium OCS (octopine synthase) promoter, the ubiquitin promoter (Holtorf S et al. (1995) Plant Mol Biol 29:637-649), the ubiquitin 1 promoter (Christensen et al. (1992) Plant Mol Biol 18:675-689; Bruce et al. (1989) Proc Natl Acad Sci USA 86:9692-9696), the Smas promoter, the cinnamyl alcohol dehydrogenase promoter (US 5,683,439), the promoters of the vacuolar ATPase subunits or the promoter of a proline-rich protein from wheat (WO 91/13991), and further promoters of genes whose constitutive expression in plants is known to the skilled worker. Especially preferred as constitutive promoter is the promoter of the nitrilase-1 (nit1) gene from A. thaliana (GenBank Acc. No.: Y07648.2, Nukleotide 2456-4340, Hillebrand et al. (1996) Gene 170:197-200).

b) Tissue-specific promoters

Preferred are furthermore promoters with specificity for the leaves, stems, roots and seeds.

Seed-specific promoters such as, for example, the phaseolin promoter (US 5,504,200; Bustos MM et al. (1989) Plant Cell 1(9):839-53; e.g. the Phaseolus vulgari; van der Geest et al. (1996) Plant Mol Biol 32:579-588), the 2S albumin promoter (Joseffson LG et al. (1987) J Biol Chem 262:12196-12201), the legumin promoter (Shirsat A et al. (1989) Mol Gen Genet

215(2): 326-331), the USP (unknown seed protein) promoter (Bäumlein H et al. (1991) Mol Gen Genet 225(3):459-467; Phillips et al. (1997) EMBO J 16:4489-4496), the napin gene promoter (US 5,608,152; Stalberg K et al. (1996) L Planta 199:515-519), the sucrose binding protein promoter (WO 00/26388), the hordein promoter (Brandt et al. (1985) Carlsberg Res. Commun. 50:333-345) or the legumin B4 promoter (LeB4; Bäumlein H et al. (1991) Mol Gen Genet 225: 121-128; Bäumlein H et al. (1992) Plant J 2(2):233-239; Fiedler U et al. (1995) Biotechnology (NY) 13(10):1090f), the Arabidopsis oleosin promoter (WO 98/45461), the Brassica Bce4 promoter (WO 91/13980).

Further suitable seed-specific promoters are those of the genes encoding the high-molecular-weight glutenin (HMWG), gliadin, branching enzyme, ADP glucose pyrophosphatase (AGPase), the napin promoter, the ACP promoter and the FatB3 and FatB4 promoters, the starch synthase promoter or the promoter of other starch-forming/modifying enzymes such as, for example, promoters of genes which encode branching enzymes (WO 92/14827, WO 92/11375). Furthermore preferred promoters are those which permit seed-specific expression in monocots such as maize, barley, wheat, rye, rice and the like. The following can be employed advantageously: the promoter of the lpt2 or lpt1 gene (WO 95/15389, WO 95/23230) or the promoters described in WO 99/16890 (promoters of the hordein gene, the glutelin gene, the oryzin gene, the prolamin gene, the gliadin gene, the glutelin gene, the zein gene, the kasirin gene, or the secalin gene). Further seed-specific promoters are described in WO 89/03887.

- tuber-, storage-root- or root-specific promoters such as, for example, the patatin promoter class I (B33), the potato Cathepsin D inhibitor promoter.
  Especially preferred in this context is the B33 promoter of the Solanum tuberosum class I patatin gene (Rocha-Sosa et al. (1989) EMBO J 8:23-29). The promoter of the class I patatin gene is approximately 100 to 1000 times more active in tubers than in leaves (Rocha-Sosa et al., vide supra). Other genes with tuber-specific expression, or at least enhanced expression in tubers, are known (for example the promoter of the ADP-glucose pyrophosphorylase genes; Müller et al. (1990) Mol Gen Genet 224:136-146).

- Leaf-specific promoters such as the potato cytosolic FBPase promoter (WO 97/05900), the Rubisco (ribulose-1,5-bisphosphate carboxylase) SSU (small

subunit) promoter (US 4,962 028) or the ST-LSI promoter from potato (Stockhaus et al. (1989) EMBO J 8:2445-2451). Very especially preferred are epidermis-specific promoters such as, for example, the OXLP gene (oxalate-oxidase-like protein) promoter (Wei et al. (1998) Plant Mol Biol 36:101-112).

c) Chemically inducible promoters

The transgenic expression constructs can also comprise a chemically inducible promoter (review article: Gatz et al. (1997) Annu Rev Plant Physiol Plant Mol Biol 48:89-108), by which the expression of the exogenous gene in the plant at a particular point in time can be controlled. Such promoters such as, for example, the PRP1 promoter (Ward et al. (1993) Plant Mol Biol 22:361-366), a salicylic-acid-inducible promoter (WO 95/19443), a benzenesulfonamide-inducible promoter (EP 0 388 186), a tetracyclin-inducible promoter (Gatz et al. (1992) Plant J 2:397-404), an abscisic-acid-inducible promoter (EP 0 335 528) or an ethanol- or cyclohexanone-inducible promoter (WO 93/21334) can likewise be used.

d) Development-dependent promoters

Further suitable promoters are, for example, fruit-maturation-specific promoters such as, for example, the tomato fruit-maturation-specific promoter (WO 94/21794, EP 409 625). Development-dependent promoters comprise partly the tissue-specific promoters, since individual tissues develop by nature in a development-dependent fashion.

e) Stress- or pathogen-inducible promoters

Further preferred promoters are those which are induced by biotic or abiotic stress such as, for example, the pathogen-inducible promoter of the PRP1 gene (Ward et al. (1993) Plant Mol Biol 22:361-366), the tomato high-temperature-inducible hsp70 or hsp80 promoter (US 5,187,267), the potato low-temperature-inducible alpha-amylase promoter (WO 96/12814) or the light-inducible PPDK promoter.

Pathogen-inducible promoters comprise the promoters of genes which are induced as a consequence of infection by pathogens, such as, for example, genes of PR proteins, SAR proteins, β-1,3-glucanase, chitinase and the like (for example Redolfi

et al. (1983) Neth J Plant Pathol 89:245-254; Uknes et al. (1992) Plant Cell 4:645-656; Van Loon (1985) Plant Mol Viral 4:111-116; Marineau et al. (1987) Plant Mol Biol 9:335-342; Matton et al. (1987) Molecular Plant-Microbe Interactions 2:325-342; Somssich et al. (1986) Proc Natl Acad Sci USA 83:2427-2430; Somssich et al. (1988) Mol Gen Genetics 2:93-98; Chen et al. (1996) Plant J 10:955-966; Zhang and Sing (1994) Proc Natl Acad Sci USA 91:2507-2511; Warner, et al. (1993) Plant J 3:191-201; Siebertz et al. (1989) Plant Cell 1:961-968(1989).

Also comprised are wounding-inducible promoters such as that of the pinII gene (Ryan (1990) Ann Rev Phytopath 28:425-449; Duan et al. (1996) Nat Biotech 14:494-498), of the wun1 and wun2 gene (US 5,428,148), of the win1 and win2 gene (Stanford et al. (1989) Mol Gen Genet 215:200-208), of the systemin gene (McGurl et al. (1992) Science 225:1570-1573), of the WIP1 gene (Rohmeier et al. (1993) Plant Mol Biol 22:783-792; Eckelkamp et al. (1993) FEBS Letters 323:73-76), of the MPI gene (Corderok et al. (1994) Plant J 6(2):141-150) and the like.

Especially preferred are promoters which are induced specifically in feeder cell systems (syncytia) following infection by nematodes. The following may be mentioned by way of example:

i) the tobacco Δ0.3 TobRB7 promoter (Opperman et al. (1994) Science 263: 221-223), in particular the promoter described by SEQ ID NO: 24,

ii) the tomato Lemmi9 promoter (Escobar et al. (1999) Mol Plant Microbe Interact 12: 440-449), in particular the promoter described by SEQ ID NO: 23, and

iii) geminivirus V sense promoters (WO 00/01832), in particular the promoters described by SEQ ID NO: 32, 33 or 34.

Further nematode-inducible promoters which are preferred for the purposes of the present invention are described in WO 98/22599. Particularly preferred in this context are the regulatory regions (i.e. the regions which precede the ATG start codon) of the sequences with the GenBank Acc. No.: A91914 (base pairs 1 to 3480). Furthermore preferred are the promoter sequences described in US 6,395,963, the promoter sequences described in WO 03/033651, the promoter sequences described in JP 2001508661-A (in particu-

lar the sequence with the GenBank Acc. No.: BD056958), and the promoter sequences described in WO 97/46692 (in particular the sequence with the GenBank Acc. No.: A79355; base pairs 1 to 2127, or 1 to 2160). Further nemotode-inducible promoters can be derived from genes whose induction as the result of nematode infection has been described. Examples which may be mentioned, but not by limitation, are: the pollenin promoter (Karimi M et al. (2002) J Nematol 34(2):75-79) and the promoter of a putative receptor serine/threonine protein kinase (Custers JHHV et al. (2002) Mol Plant Pathol 3(4):239-249).

Especially preferred are pathogen- or stress-inducible promoters and seed-, tuber-, root-, leaf- and/or stem-specific promoters, with pathogen-inducible promoters (in particular the nematode-inducible promoters which have been mentioned individually above) being most preferred.

A further - especially preferred - subject of the invention relates to expression constructs in which a nucleic acid sequence encoding a protein with sucrose isomerase activity is in operable linkage with a stress-, pathogen-, or wounding-inducible promoter. Stress-, pathogen- or wounding-inducible promoters generally refers to all those promoters which are capable of being induced by biotic or abiotic stress. Abiotic stress means, in this context, stimuli such as high and low temperatures, dryness, frost, humidity, salt, UV light and the like. Biotic stress means, in this context, the infection with a pathogen, the term "pathogen" comprising all the abovementioned pathogens. This stimulus is preferably potent enough to lead to a yield reduction of at least 5% in comparison with average yields. Inducible means, in this context, an increase of the transcription activity by at least 50%, preferably at least 100%, especially preferably at least 500%, very especially preferably at least 1000%, most preferably at least 5000% in comparison with the expression activity of a nonstimulated plant. By way of example, but not by limitation, stress- or pathogen-inducible promoters comprise the pathogen-inducible promoter of the PRP1 gene (Ward et al. (1993) Plant Mol Biol 22:361-366), the tomato high-temperature-inducible hsp70 or hsp80 promoter (US 5,187,267), the potato low-temperature-inducible α-amylase promoter (WO 96/12814), the light-inducible PPDK promoter or the wounding-inducible pinII promoter (EP-A 0 375 091). Preferred are, in particular, pathogen-inducible promoters such as, for example, the promoters of the PR proteins, SAR proteins, β-1,3-glucanase, chitinase and the like (for example Redolfi et al. (1983) Neth J Plant Pathol 89:245-254; Uknes, et al. (1992) The Plant Cell 4:645-656; Van Loon (1985) Plant Mol Viral 4:111-116; Marineau et al. (1987)

Plant Mol Biol 9:335-342; Matton et al. (1987) Molecular Plant-Microbe Interactions 2:325-342; Somssich et al. (1986) Proc Natl Acad Sci USA 83:2427-2430; Somssich et al. (1988) Mol Gen Genetics 2:93-98; Chen et al. (1996) Plant J 10:955-966; Zhang and Sing (1994) Proc Natl Acad Sci USA 91:2507-2511; Warner, et al. (1993) Plant J 3:191-201; Siebertz et al. (1989) Plant Cell 1:961-968). Also comprised are wounding-inducible promoters such as that of the pinII gene (Ryan (1990) Ann Rev Phytopath 28:425-449; Duan et al. (1996) Nat Biotech 14:494-498), of the wun1 and wun2 gene (US 5,428,148), of the win1 and win2 gene (Stanford et al. (1989) Mol Gen Genet 215:200-208), of the systemin gene (McGurl et al. (1992) Science 225:1570-1573), of the WIP1 gene (Rohmeier et al. (1993) Plant Mol Biol 22:783-792; Eckelkamp et al. (1993) FEBS Letters 323:73-76), of the MPI gene (Corderok et al. (1994) Plant J 6(2):141-150) and the like. Wounding-inducible promoters can be employed advantageously in the case of infection by feeding pathogens.

Moreover, the average skilled worker can additionally readily find more examples of genes with stress-, pathogen- or wounding-induced expression patterns in the literature. Furthermore, the average skilled worker is capable of isolating further suitable promoters by means of routine methods. Thus, the skilled worker can identify suitable regulatory nucleic acid elements with the aid of conventional molecular-biological methods, for example hybridization experiments or DNA-protein-binding studies. In this context, a first step may consist in establishing a differential expression library of, for example, pathogen-infected/infested and "normal" tissue. Thereafter, promoters which have pathogen-inducible regulatory elements are isolated with the aid of the pathogen-induced cDNAs thus identified. In addition, the skilled worker has available further, PCR-based methods for the isolation of suitable stress-, pathogen- or wounding-induced promoters.

Especially preferred are tissue-specific promoters, in particular seed-specific, tuber-specific, fruit-specific and leaf-specific promoters, and pathogen-induced promoters.
Very especially preferred are pathogen-induced promoters, in particular nematode-induced promoters.

Furthermore, further promoters may be linked operably to the nucleic acid sequence to be expressed, which promoters make possible the recombinant expression in further plant tissues or in other organisms, such as, for example, *E. coli* bacteria. Suitable plant promoters are, in principle, all of the above-described promoters.

The nucleic acid sequences present in the expression constructs or expression vectors can be linked operably to further genetic control sequences in addition to a promoter. The term "genetic control sequences" is to be understood in the broad sense and refers to all those sequences which have an effect on the materialization or the function of an expression construct. For example, genetic control sequences modify the transcription and translation in prokaryotic or eukaryotic organisms. Preferably, the expression constructs comprise a plant-specific promoter 5'-upstream of the nucleic acid sequence in question to be expressed recombinantly, and 3'-downstream a terminator sequence as additional genetic control sequence and, if appropriate, further customary regulatory elements, in each case linked operably to the nucleic acid sequence to be expressed recombinantly.

Genetic control sequences also comprise further promoters, promoter elements or minimal promoters, all of which can modify the expression-governing properties. Thus, for example, the tissue-specific expression may additionally depend on certain stress factors, owing to genetic control sequences. Such elements have been described, for example, for water stress, abscisic acid (Lam E and Chua NH (1991) J Biol Chem 266(26): 17131-17135) and heat stress (Schoffl F et al. (1989) Mol Gen Genetics 217(2-3):246-53).

Genetic control sequences furthermore also comprise the 5'-untranslated regions, introns or noncoding 3'-region of genes, such as, for example, the actin-1 intron, or the Adh1-S introns 1, 2 and 6 (general reference: The Maize Handbook, Chapter 116, Freeling and Walbot, Eds., Springer, New York (1994)). It has been demonstrated that they may play a significant role in the regulation of gene expression. Thus, it has been demonstrated that 5'-untranslated sequences can enhance the transient expression of heterologous genes. Examples of translation enhancers which may be mentioned are the tobacco mosaic virus 5' leader sequence (Gallie et al. (1987) Nucl Acids Res 15:8693-8711) and the like. Furthermore, they may promote tissue specificity (Rouster J et al. (1998) Plant J 15:435-440).

The transgenic expression construct may advantageously comprise one or more of what are known as enhancer sequences, linked operably to the promoter, which make possible an increased recombinant expression of the nucleic acid sequence. Additional advantageous sequences, such as further regulatory elements or terminators, may also be inserted at the 3' end of the nucleic acid sequences to be expressed recombinantly. One or more copies

of the nucleic acid sequences to be expressed recombinantly may be present in the gene construct.

Polyadenylation signals which are suitable as control sequences are plant polyadenylation signals, preferably those which essentially correspond to T-DNA polyadenylation signals from *Agrobacterium tumefaciens*. Examples of terminator sequences which are especially suitable are the OCS (octopine synthase) terminator and the NOS (nopaline synthase) terminator.

Control sequences are furthermore to be understood as those which make possible homologous recombination or insertion into the genome of a host organism or which permit removal from the genome. Upon homologous recombination, for example the coding sequence of a particular endogenous gene may be specifically exchanged for the sequence which encodes a sucrose isomerase.

A transgenic expression construct and/or the transgenic expression vectors derived from it may comprise further functional elements. The term functional element is to be understood in the broad sense and refers to all those elements which have an effect on the generation, amplification or function of the transgenic expression constructs, the transgenic expression vectors or the transgenic organisms according to the invention. The following may be mentioned by way of example, but not by limitation:

a) Selection markers which confer a resistance to biocides, e.g. metabolism inhibitors (such as 2-deoxyglucose-6-phosphate (WO 98/45456)), antibiotics (such as, for example, kanamycin, G 418, bleomycin or hygromycin) or herbicides (such as glyphosate or phosphinothricin).

Especially preferred selection markers are those which confer resistance to herbicides. Examples which may be mentioned are: DNA sequences which encode phosphinothricin acetyl transferases (PAT) and which inactivate glutamin synthase inhibitors (bar and pat genes), 5-enolpyruvylshikimate-3-phosphate synthase genes (EPSP synthase genes), which confer resistance to Glyphosate® (N-(phosphonomethyl)glycine), the gox gene, which encodes Glyphosate®-degrading enzymes (glyphosate oxidoreductase), the deh gene (encoding a dehalogenase which inactivates dalapon), sulfonylurea- and imidazolinone-inactivating acetolactate synthases, and bxn genes, which encode bromoxynil-degrading nitrilase enzymes, the aasa gene, which confers resistance to the antibiotic apectinomycin, the

streptomycin phosphotransferase (spt) gene, which allows resistance to streptomycin, the neomycin phosphotransferase (nptII) gene, which confers resistance to kanamycin or geneticin (G418), the hygromycin phosphotransferase (hpt) gene, which mediates resistance to hygromycin, the acetolactate synthase gene (als), which confers resistance to sulfonylurea herbicides (for example mutated ALS variants with, for example, the S4 and/or Hra mutation).

b) Reporter genes which encode readily quantifiable proteins and, via their color or enzyme activity, make possible an assessment of the transformation efficacy, the site of expression or the time of expression. Very especially preferred in this context are reporter proteins (Schenborn E, Groskreutz D. Mol Biotechnol. 1999; 13(1):29-44) such as the green fluorescent protein (GFP) (Sheen et al.(1995) Plant Journal 8(5):777-784), chloramphenicol transferase, luciferase (Ow et al. (1986) Science 234:856-859), aequorin (Prasher et al. (1985) Biochem Biophys Res Commun 126(3):1259-1268), β-galactosidase, with β-glucuronidase being very especially preferred (Jefferson et al. (1987) EMBO J 6:3901-3907).

c) Origins of replication, which ensure amplification of the transgenic expression constructs or transgenic expression vectors according to the invention in, for example, E. coli. Examples of ORI (origin of DNA replication) which may be mentioned are the pBR322 ori or the P15A ori (Sambrook et al.: Molecular Cloning. A Laboratory Manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989).

d) Elements which are necessary for Agrobacterium-mediated plant transformation, such as, for example, the right or left border of the T-DNA or the vir region.

To select successfully transformed cells, it is, as a rule, necessary additionally to introduce a selectable marker which confers resistance to a biocide (for example a herbicide), to a metabolism inhibitor such as 2-deoxyglucose-6-phosphate (WO 98/45456) or to an antibiotic to the cells which have successfully undergone recombination. The selection marker permits the selection of the transformed cells from untransformed ones (McCormick et al. (1986) Plant Cell Reports 5:81-84).

The introduction of an expression construct according to the invention into an organism or cells, tissues, organs, parts or seeds thereof (preferably into plants or plant cells, tissue, organs, parts or seeds) can be effected advantageously using vectors which comprise the transgenic expression constructs. Examples of vectors may be plasmids, cosmids, phages, viruses or else agrobacteria. The transgenic expression construct can be introduced into the vector (preferably a plasmid vector) via a suitable restriction cleavage site or a recombinase att sequence. The transgenic expression vector formed is first introduced into E. coli. Correctly transformed E. coli are selected, grown, and the recombinant vector is obtained by the methods familiar to the skilled worker. Restriction analysis and sequencing may serve to verify the cloning step. Preferred vectors are those which make possible stable integration of the transgenic expression contruct into the plant genome.

The generation of a transformed organism (or of a transformed cell or tissue) requires introducing the DNA (for example the expression vector) or RNA in question into the relevant host cell. A multiplicity of methods are available for this procedure, which is termed transformation (or transduction or transfection) (Keown et al. (1990) Methods in Enzymology 185:527-537). For example, the DNA or RNA can be introduced directly by microinjection or by bombardment with DNA-coated microparticles. Also, the cell can be permeabilized chemically, for example using polyethylene glycol, so that the DNA can enter the cell by diffusion. The DNA can also be introduced by protoplast fusion with other DNA-containing units such as minicells, cells, lysosomes or liposomes. Another suitable method of introducing DNA is electroporation, where the cells are permeabilized reversibly by an electrical pulse. Suitable methods have been described (for example by Bilang et al. (1991) Gene 100:247-250; Scheid et al. (1991) Mol Gen Genet 228:104-112; Guerche et al. (1987) Plant Science 52:111-116; Neuhause et al. (1987) Theor Appl Genet 75:30-36; Klein et al. (1987) Nature 327:70-73; Howell et al. (1980) Science 208:1265; Horsch et al.(1985) Science 227:1229-1231; DeBlock et al. (1989) Plant Physiology 91:694-701; Methods for Plant Molecular Biology (Weissbach and Weissbach, eds.) Academic Press Inc. (1988); and Methods in Plant Molecular Biology (Schuler and Zielinski, eds.) Academic Press Inc. (1989)).

In plants, the above-described methods of transforming and regenerating plants from plant tissues or plant cells are exploited for transient or stable transformation. Suitable methods are especially protoplast transformation by

polyethylene-glycol-induced DNA uptake, the biolistic method with the gene gun, what is known as the particle bombardment method, electroporation, incubation of dry embryos in DNA-containing solution, and microinjection.

In addition to these "direct" transformation techniques, transformation can also be effected by bacterial infection by means of Agrobacterium tumefaciens or Agrobacterium rhizogenes. The Agrobacterium-mediated transformation is best suited to dicotyledonous plant cells. The methods are described, for example, by Horsch RB et al. (1985) Science 225: 1229f.

When agrobacteria are used, the transgenic expression construct must be integrated into specific plasmids, either into a shuttle or intermediate vector, or into a binary vector. If a Ti or Ri plasmid is to be used for the transformation, at least the right border, but in most cases the right and left border, of the Ti or Ri plasmid T-DNA is linked to the transgenic expression construct to be introduced in the form of a flanking region.

Binary vectors are preferably used. Binary vectors are capable of replication both in E. coli and in Agrobacterium. As a rule, they comprise a selection marker gene for the selection of transformed plants (for example the nptII gene, which confers resistance to kanamycin) and a linker or polylinker flanked by the right and left T-DNA border sequence. Apart from the T-DNA border sequence, they additionally comprise a selection marker which permits the selection of transformed E. coli and/or agrobacteria (e.g. the nptIII gene, which confers resistance to kanamycin). Such vectors can be transformed directly into Agrobacterium (Holsters et al. (1978) Mol Gen Genet 163:181-187).

The Agrobacterium which acts as host organism in this case should already contain a plasmid with the vir region. The latter is required for transferring the T-DNA to the plant cell. An Agrobacterium transformed in this way can be used for transforming plant cells. The use of T-DNA for transforming plant cells has been studied and described intensively (EP 120 516; Hoekema, In: The Binary Plant Vector System, Offsetdrukkerij Kanters B.V., Alblasserdam, Chapter V; An et al. (1985) EMBO J 4:277-287). Various binary vectors are known, some of which are commercially available such as, for example, pBI101.2 or pBIN19 (Clontech Laboratories, Inc. USA).

Direct transformation techniques are suitable for any organism and cell type. The plasmid used need not meet any particular requirements in the case of the injection or electroporation of

DNA or RNA into plant cells. Simple plasmids such as those of the pUC series can be used. If complete plants are to be regenerated from the transformed cells, it is advantageous for an additional selectable marker gene to be located on the plasmid.

Stably transformed cells, i.e. those which contain the introduced DNA integrated into the DNA of the host cell, can be selected from untransformed cells when a selectable marker is part of the DNA introduced. Examples of genes which can act as markers are all those which are capable of conferring resistance to antibiotics or herbicides (such as kanamycin, G 418, bleomycin, hygromycin or phosphinothricin etc.) (see above). Transformed cells which express such marker genes are capable of surviving in the presence of concentrations of a corresponding antibiotic or herbicide which kill an untransformed wild type. Examples are mentioned above and preferably comprise the bar gene, which confers resistance to the herbicide phosphinothricin (Rathore KS et al. (1993) Plant Mol Biol 21(5):871-884), the nptII gene, which confers resistance to kanamycin, the hpt gene, which confers resistance to hygromycin, or the EPSP gene, which confers resistance to the herbicide glyphosate. The selection marker permits the selection of transformed cells from untransformed cells (McCormick et al. (1986) Plant Cell Reports 5:81-84). The resulting plants can be bred and hybridized in the customary fashion. Two or more generations should be grown in order to ensure that the genomic integration is stable and hereditary.

The abovementioned methods are described, for example, in Jenes B et al.(1993) Techniques for Gene Transfer, in: Transgenic Plants, Vol. 1, Engineering and Utilization, edited by SD Kung and R Wu, Academic Press, pp. 128-143 and in Potrykus (1991) Annu Rev Plant Physiol Plant Molec Biol 42:205-225. The expression construct is preferably cloned into a vector which is suitable for the transformation of Agrobacterium tumefaciens, for example pBin19 (Bevan et al. (1984) Nucl Acids Res 12:8711f).

As soon as a transformed plant cell has been generated, a complete plant can be obtained using methods known to the skilled worker. For example, callus cultures are used as starting material. The development of shoot and root can be induced from this as yet undifferentiated cell biomass in a known fashion. The shoots obtained can be planted out and bred.

The skilled worker is familiar with such methods of regenerating intact plants from plant cells and plant parts. Methods to do so are described, for example, by Fennell et al. (1992) Plant Cell

Rep. 11: 567-570; Stoeger et al (1995) Plant Cell Rep. 14:273-278; Jahne et al. (1994) Theor Appl Genet 89:525-533.

"Transgenic/recombinant" refers to all those constructs and methods in which either

a) the nucleic acid sequence encoding a protein with sucrose isomerase activity, or

b) a genetic control sequence linked operably to said nucleic acid sequence under a), for example a promoter, or

c) (a) and (b)

are not located in their natural genetic environment or have been modified by recombinant methods, an example of a modification being a substitution, addition, deletion, inversion or insertion of one or more nucleotide residues. Natural genetic environment refers to the natural chromosomal locus in the organism of origin, or to the presence in a genomic library.

Sequences

1. SEQ ID NO: 1 nucleic acid sequence encoding *Protaminobacter rubrum* sucrose isomerase

2. SEQ ID NO: 2 amino acid sequence encoding *Protaminobacter rubrum* sucrose isomerase

3. SEQ ID NO: 3 nucleic acid sequence encoding sucrose isomerase of *Erwinia rhaponthici* sucrose isomerase (N-terminal fragment)

4. SEQ ID NO: 4 amino acid sequence encoding sucrose isomerase of *Erwinia rhaponthici* sucrose isomerase (N-terminal fragment)

5. SEQ ID NO: 5 nucleic acid sequence encoding *Erwinia rhaponthici* sucrose isomerase

6. SEQ ID NO: 6 amino acid sequence encoding *Erwinia rhaponthici* sucrose isomerase

7. SEQ ID NO: 7 nucleic acid sequence encoding *Protaminobacter rubrum* sucrose isomerase (variant)

8. SEQ ID NO: 8 amino acid sequence encoding *Protaminobacter rubrum* sucrose isomerase (variant)

9. SEQ ID NO: 9 nucleic acid sequence encoding *Enterobacter species SZ62* sucrose isomerase

10. SEQ ID NO: 10 amino acid sequence encoding *Enterobacter species SZ62* sucrose isomerase

11. SEQ ID NO: 11 nucleic acid sequence encoding *Serratia plymuthica* sucrose isomerase

12. SEQ ID NO: 12 amino acid sequence encoding *Serratia plymuthica* sucrose isomerase

13. SEQ ID NO: 13 nucleic acid sequence encoding fusion protein of *Erwinia rhapontici* sucrose isomerase (*palI*) and signal peptide sequence of the proteinase inhibitor II gene

14. SEQ ID NO: 14 amino acid sequence encoding fusion protein of *Erwinia rhapontici* (*palI*) sucrose isomerase and signal peptide sequence of the proteinase inhibitor II gene

15. SEQ ID NO: 15 nucleic acid sequence (complete cDNA with untranslated region) encoding Klebsiella sp. LX3 sucrose isomerase (isomaltulose synthase)

16. SEQ ID NO: 16 amino acid sequence encoding Klebsiella sp. LX3 sucrose isomerase (isomaltulose synthase)

17. SEQ ID NO: 17 nucleic acid sequence (open reading frame) encoding Klebsiella sp. LX3 sucrose isomerase (isomaltulose synthase)

18. SEQ ID NO: 18 amino acid sequence encoding Klebsiella sp. LX3 sucrose isomerase (isomaltulose synthase)

19. SEQ ID NO: 19 nucleic acid sequence encoding Enterobacter species SZ62 sucrose isomerase (fragment)

20. SEQ ID NO: 20 amino acid sequence encoding Enterobacter species SZ62 sucrose isomerase (fragment)

21. SEQ ID NO: 21 nucleic acid sequence encoding Pseudomonas mesoacidophila MX45 sucrose isomerase (fragment)

22. SEQ ID NO: 22 amino acid sequence encoding Pseudomonas mesoacidophila MX45 sucrose isomerase (fragment)

23. SEQ ID NO: 23 nucleic acid sequence encoding tomato (Lycopersicum esculentum) Lemmi9 promoter

24. SEQ ID NO: 24 nucleic acid sequence encoding Nicotiana tabacum Δ0..3TobRB7 promoter sequence (Region: -298 to +76)

25. SEQ ID NO: 25 oligonucleotide primer FB83
5'-GGATCCGGTACCGTTCAGCAATCAAAT-3'

26. SEQ ID NO: 26 oligonucleotide primer FB84
5'-GTCGACGTCTTGCCAAAAACCTT-3'

27. SEQ ID NO: 27 oligonucleotide primer FB 97
5'-GTCGACCTACGTGATTAAGTTTATA-3'

28. SEQ ID NO: 28 oligonucleotide primer Lem1
5'-atcGAATTCATAATTTAACCATCTAGAG-3'

29. SEQ ID NO: 29 oligonucleotide primer Lem2
5'-atcGGTACCTGCTTCTGGAACGAAAGGG-3'

30. SEQ ID NO: 30 oligonucleotide primer Tob1
5'-GGAATTCAGCTTATCTAAACAAAGTTTTAAATTC-3'

31. SEQ ID NO: 31 oligonucleotide primer Tob2
5'-GGGTACCAGTTCTCACTAGAAAAATGCCCC-3

32. SEQ ID NO: 32 nucleic acid sequence encoding wheat dwarf virus V-sense promoter (Genbank Acc. No.: AX006849; sequence 1 in WO 00/01832)

33. SEQ ID NO: 33 nucleic acid sequence encoding maize streak virus V-sense promoter (GenBank Acc. No.: AX006850; sequence 2 in WO 00/01832)

34. SEQ ID NO: 34 nucleic acid sequence encoding pepper hausteco virus V-sense promoter (GenBank Acc. No.: AX006851; sequence 3 in WO 00/01832)

35. SEQ ID NO: 35 nucleic acid sequence encoding Serratia plymuthica sucrose isomerase

36. SEQ ID NO: 36 amino acid sequence encoding Serratia plymuthica sucrose isomerase

Figures

1. Fig. 1: Schematic representation of the expression cassette in the plasmid p35S-cwIso. The abbreviations denote:
   35S: 35S cauliflower mosaic virus (CaMV) promoter
   SP: signal peptide of the proteinase inhibitor II gene
   palI: Erwinia rhapontici sucrose isomerase gene
   OCS: polyadenylation signal of the octopine synthase gene
   EcoRI,Asp718,BamHI,SalI,HindIII: restriction cleavage sites
   Detailed description of the individual elements, see hereinbelow.

2. Fig 2: Schematic representation of the expression cassette in the plasmid pB33-cwIso. The abbreviations denote:
   B33: promoter of the class I patatin gene B33
   SP: signal peptide of the proteinase inhibitor II gene
   palI: Erwinia rhapontici sucrose isomerase gene
   OCS: polyadenylation signal of the octopine synthase gene
   EcoRI,Asp718,BamHI,SalI,HindIII: restriction cleavage sites
   Detailed description of the individual elements, see hereinbelow.

3. Fig. 3: Western blot analysis of palI-expressing potato tubers of various transgenic lines. 20 µg of soluble protein were applied to each lane of an SDS gel, separated and transferred to nitrocellulose. The filters were subsequently hybridized with a polyclonal PalI antibody. The expression in tubers from wild-type potato plants (wt) was compared with the expression in potato lines 5, 12, 26 and 33.

4. Fig. 4: HPLC analysis of the soluble carbohydrates in sucrose-isomerase-expressing plants.
   A: Sugar standards.
   B: Extract from a transgenic tuber.
   C: Extract from a wild-type tuber.

5. Fig. 5: Palatinose, sucrose, glucose and starch content in wild-type potato tubers (wt) and in potato tubers from various transgenic lines (3 to 37), which express the chimeric palI gene in the cell wall. The data of the wild type (wt; hatched columns) and the transgenic potato tubers (3 to 37; black columns) correspond to the means of four measurements ± standard deviation. A transgenic, but not *palI*-expressing, line (control) was analyzed as additional control.

6. Fig.6: Infection of potato tubers with Alternaria solani. Potato disks from wild-type tubers and tubers of the palI-expressing transgenic lines 5 and 33 14 days post-infection with Alternaria solani.
   A: Control with potato disks of wild-type (wt) and transgenic tubers (lines 5 and 33) after incubation for 14 days without previous Alternaria-infection.
   B: Wild-type tubers; 14 days after Alternaria-infection
   C: Transgenic line 5; 14 days after Alternaria-infection
   D: Transgenic line 33; 14 days after Alternaria-infection

7. Fig. 7: Schematic representation of the expression cassette in the plasmid pLemmi9-cwIso. The abbreviations denote:
   Lemmi9: tomato (Lycopersicon esculentum) Lemmi9 promoter
   SP: signal peptide of the proteinase inhibitor II gene
   palI: Erwinia rhapontici sucrose isomerase gene
   OCS: polyadenylation signal of the octopine synthase gene
   EcoRI,Asp718,BamHI,SalI,HindIII: restriction cleavage sites
   Detailed description of the individual elements, see hereinbelow.

8. Fig 8: Schematic representation of the expression cassette in the plasmid pΔ0.3TobRB7-cwIso. The abbreviations denote:
   Δ0.3TobRB: Nicotiana tabacum Δ0.3TobRB7 promoter
   SP: signal peptide of the proteinase inhibitor II gene
   palI: Erwinia rhapontici sucrose isomerase gene
   OCS: polyadenylation signal of the octopine synthase gene
   EcoRI,Asp718,BamHI,SalI,HindIII: restriction cleavage sites
   Detailed description of the individual elements, see hereinbelow.

Examples

General methods:

The chemical synthesis of oligonucleotides can be effected, for example, in the known fashion using the phosphoamidite method (Voet, Voet, 2nd Edition, Wiley Press New York, pages 896-897). The cloning steps carried out for the purposes of the present invention - such as, for example, restriction cleavages, agarose gel electrophoresis, purification of DNA fragments, transfer of nucleic acids to nitrocellulose and nylon membranes, linking DNA fragments, transformation of E. coli cells, growing bacteria, multiplying phages and sequence analysis of recombinant DNA - are carried out as described by Sambrook et al. (1989) Cold Spring Harbor Laboratory Press; ISBN 0-87969-309-6. The transformation of Agrobacterium tumefaciens was carried out in accordance with

the method of Hofgen and Willmitzer ((1988) Nucl. Acids Res. 16:9877). The Agrobacteria were grown in YEB medium (Vervliet et al. (1975) Gen Virol 26:33). The sequencing of recombinant DNA molecules is carried out with an MWG-Licor laser fluorescence DNA sequencer following the method of Sanger (Sanger et al. (1977) Proc Natl Acad Sci USA 74:5463-5467).

Example 1: PCR amplification of a subfragment of the Erwinia rhapontici sucrose isomerase

A subfragment of the sucrose isomerase was cloned by means of polymerase chain reaction (PCR). The template material was genomic DNA from E. rhapontici (DSM 4484), which was isolated by a standard protocol. Amplification was carried out using the following specific primers, which were derived from a prior-art sucrose isomerase sequence:

FB83 5'-GGATCCGGTACCGTTCAGCAATCAAAT-3' (SEQ ID NO: 25)

FB84 5'-GTCGACGTCTTGCCAAAAACCTT-3' (SEQ ID NO: 26)

Primer FB83 comprises the bases 109 to 127 and primer FB84 the bases 1289 to 1306 of the coding region of the E. rhapontici sucrose isomerase gene.

The PCR reaction mixture (100 µl) comprised:

- chromosomal bacterial DNA (1 µg)
- primers FB 83 and FB 84 (250 ng each),
- Pfu DNA polymerase reaction buffer (10 µl, Stratagene),
- 200 µM dNTPs (dATP, dCTP, dGTP, dTTP) and
- 2.5 units Pfu DNA polymerase (Stratagene).

Before starting the amplification cycles, the mixture was heated for 5 minutes at 95°C. The polymerization steps (30 cycles) were carried out in an automatic T3 thermocycler (Biometra) with the following program: denaturation 95°C (1 minute), annealing of the primers at 55°C (40 seconds), polymerase reaction at 72°C (2 minutes). The resulting fragment was cloned into the vector pCR-Blunt (Invitrogen). The identity of the amplified DNA was verified by means of sequence analysis.

The subfragment which has been amplified can also be employed as hybridization probe for the isolation of further sucrose isomerase DNA sequences from other organisms or as probe in the analysis of transgenic cells and plants.

Example 2: PCR amplification of an Erwinia rhapontici sucrose isomerase

Using the fragment amplified in Example 1, a genomic Erwinia rhapontici library was screened by standard methods. Subsequent sequence analysis permitted the determination of the open reading frame of sucrose isomerase. The oligonucleotide primers FB83 and FB97 were derived from this sequence.

The complete open reading frame of sucrose isomerase was cloned by means of polymerase chain reaction (PCR). Genomic DNA from E. rhapontici (DSM 4484) which had been isolated following a standard protocol acted as template material. The amplification was carried out using the following specific primers:

FB83 5'-GGATCCGGTACCGTTCAGCAATCAAAT-3' (SEQ ID NO: 25)

FB97 5'-GTCGACCTACGTGATTAAGTTTATA-3' (SEQ ID NO: 27)

Primer FB83 comprises the bases 109 to 127 and primer FB97 the bases 1786 to 1803 of the coding region of the sucrose isomerase gene. To clone the amplified DNA into expression vectors, the primers additionally have the following restriction cleavage sites: primer FB 83, BamHI, and primer FB 97, SalI.

The PCR reaction mixture (100 µl) comprised:

- chromosomal bacterial DNA (1 µg),
- primers FB83 and FB97 (250 ng each),
- Pfu DNA polymerase reaction buffer (10 µl, Stratagene),
- 200 µM dNTPs (dATP, dCTP, dGTP, dTTP) and
- 2.5 units Pfu DNA polymerase (Stratagene).

Before starting the amplification cycles, the mixture was heated for 5 minutes at 95°C. The polymerization steps (30 cycles) were carried out in an automatic T3 thermocycler (Biometra) with the following program: denaturation 95°C (1 minute), annealing of the primers at 55°C (40 seconds), polymerase reaction at 72°C (2 minutes). The sucrose isomerase fragment which had been amplified was cloned into the vector pCR-Blunt (Invitrogen), giving rise to the plasmid pCR-SucIso2 (without translation origin). The identity of the amplified DNA was verified by means of sequence analysis. The PCR fragment thus comprises the sequence of an E. rhapontici sucrose isomerase which extends from nucleotide 109-1803 of the sucrose isomerase gene.

Example 3: Generation of plasmid p35S-cwIso

A DNA sequence which encodes a sucrose isomerase was isolated from the plasmid pCR-SucIso2 and provided with the cauliflower mosaic virus 35S promoter, which mediates constitutive expression in transgenic plant cells, and with a plant termination signal. The plant termination signal comprises the 3' end of the polyadenylation site of the octopine synthase gene.

Moreover, a signal peptide of a plant gene (potato proteinase inhibitor II gene (Keil et al. (1986) Nucl Acids Res 14:5641-5650; Genbank Acc. No.: X04118)), which is required for the uptake into the endoplasmic reticulum, was fused upstream of the coding sequence of the sucrose isomerase gene. To this end, the sucrose isomerase fragment was excised from the construct pCR-SucIso2 via the restriction cleavage sites BamHI and SalI and ligated into a BamHI/SalI-linearized pMA vector. The vector pMA is a modified form of the vector pBinAR (Höfgen and Willmitzer (1990) Plant Sci. 66:221-230), which comprises the cauliflower mosaic virus 35S promoter, which mediates constitutive expression in transgenic plants, a signal peptide of the potato proteinase inhibitor II, which mediates targeting of the fusion protein into the cell wall, and a plant termination signal. The plant termination signal comprises the 3' end of the polyadenylation site of the octopine synthase gene. Between the part-sequence of the proteinase inhibitor and the termination signal there are located cleavage sites for the restriction enzymes BamHI, XbaI, SalI, PstI and SphI (in this order), which make possible the insertion of suitable DNA fragments so that a fusion protein between the proteinase inhibitor signal peptide and the inserted protein is formed, which is then transported into the cell wall of transgenic plant cells which express this protein. Thus, the expression cassette in the plasmid p35S-cwIso consists of the fragments A, B and C (Fig. 1):

A) Fragment A comprises the cauliflower mosaic virus 35S promoter (CaMV). It comprises a fragment comprising the nucleotides 6909 to 7437 of the CaMV (Franck (1980) Cell 21:285).

B) Fragment B comprises the nucleotides 923 to 1059 of a potato proteinase inhibitor II gene (Keil el al., supra), which are fused via a linker with the sequence ACC GAA TTG GG to the Erwinia rhapontici sucrose isomerase gene, which comprises the nucleotides 109 to 1803. Thus, a signal peptide of a plant protein, which is required for the uptake of proteins

into the endoplasmic reticulum (ER) is fused N-terminally to the sucrose isomerase sequence.

C) Fragment C comprises the polyadenylation signal of the octopine synthase gene (Dhaese et al. (1983) EMBO J. 2:419-426. GenBank Acc. No.: Z37515, nucleotides 1344 to 1533).

In p35S-cwIso (35S = 35S promoter, cw = cell wall, Iso = sucrose isomerase), the coding region of the E. rhapontici sucrose isomerase is under constitutive control, the gene product is taken up into the ER and subsequently secreted.

Example 4: Generation of plasmid pB33-cwIso

The plasmid pB33-cwIso was generated using the binary plasmid p35S-cwIso. In doing so, the 35S promoter was exchanged for the promoter of the class I patatin gene (Rocha-Sosa et al (1989) EMBO J 8:23-29). Thus, the expression cassette of this plasmid pB33-cwIso consists of the three fragments A, B and C (see Fig. 2):

A) Fragment A comprises the region -1512 to +14 relative to the transcription initiation site of the class I patatin gene. The promoter region was ligated, in the form of a DraI fragment, into the SstI-cut vector pUC18, whose ends had been filled up using T4 DNA polymerase and thus been made blunt ended. The fragment was subsequently reexcised from the vector pUC18 using the restriction enzymes EcoRI and Asp718 and cloned into the plasmid p35S-cwIso, from which the 35S CaMV promoter had previously been deleted after partial restriction with the enzymes EcoRI and Asp718.

B) Fragment B comprises the nucleotides 923 to 1059 of a potato proteinase inhibitor II gene, which are fused via a linker with the sequence ACC GAA TTG GG to the E. rhapontici sucrose isomerase gene, which comprises the nucleotides 109 to 1803. Thus, a signal peptide of a plant protein, which is required for the uptake of proteins into the ER is fused N-terminally to the sucrose isomerase sequence.

C) Fragment C comprises the polyadenylation signal of the octopine synthase gene (Dhaese et al. (1983) EMBO J 2:419-426; GenBank Acc. No.: Z37515, nucleotides 1344 to 1533).

In pB33-cwIso (B33 = promoter of the class I patatin gene B33, cw = cell wall, Iso = sucrose isomerase), the coding region of the E. rhapontici sucrose isomerase is under tissue-specific control, the gene product is taken up into the ER.

Example 5: Transformation of potatoes, and selection of transgenic plants

20 small leaves from a sterile potato culture (Solanum tuberosum L. cv. Solara), which had been wounded with a surgical blade, were placed into 10 ml of MS medium supplemented with 2% sucrose and comprising 50 µl of an Agrobacterium tumefaciens overnight culture grown under selection. After gentle shaking for 5 minutes, the Petri dishes were incubated in the dark at 25°C. After two days, the leaves were plated on MS medium supplemented with 1.6% glucose, 2 mg/l zeatin ribose, 0.02 mg/l giberellic acid, 500 mg/l claforan, 50 mg/l kanamycin and 0.8% Bacto agar. After incubation for one week at 25°C and 3000 Lux, the claforan concentration in the medium was halved. Cultivation was continued for one week under known conditions (Rocha-Sosa et al. (1989) EMBO J 8:23-29).

Using the plasmid pB33-cwIso, the Agrobacterium-mediated transformation was followed by the regeneration of a total of 36 kanamycin-resistant potato plants. The tubers of these plants were examined for palI expression with the aid of a polyclonal antibody against the recombinant PalI protein (Börnke et al. (2002) Planta 214:356-364). palI-expression was detected in a Western blot in 25 lines. A Western blot of representative lines is shown in Fig. 3.

Example 6: HPLC analysis of the transgenic pB33-cwIso potatoes

Tuber extracts of the transgenic lines were analyzed via HPLC for their soluble carbohydrate content with the purpose of detecting the in-vivo conversion of sucrose to palatinose. The HPLC analysis was carried out by the method described in Börnke et al. (2002) Planta 214:356-364. The preparation of the tuber extract is described by Sonnewald et al.(1992) Plant J 2:571-581. The results of the HPLC analysis are shown in Fig. 4.

As shown by the chromatograms, the expression of sucrose isomerase in the cell wall results in a substantial accumulation of palatinose in the tubers of the pB33-cwIso lines studied. The wild type comprises no palatinose, as can likewise be seen clearly from the chromatograms. The soluble sugar content in the transgenic potato tubers comprising the construct pB33-cwIso is shown

in Fig. 5. The palatinose content in the potato tubers varies between the individual transgenic lines between 1.7 µmol/g FW (FW: "fresh weight") (line 14) and 16 µmol/g FW (line 5).

Example 7: Infection of potato disks with Alternaria solani

Alternaria solani (provided by Dr. Jürgen Sigrist, Zentrum für Grüne Gentechnik [Center for Green Genetic Engineering], Neustadt an der Weinstraße) were maintained for 14 days at 16°C on PDA agar (Duchefa, The Netherlands) (PDA = potato dextrose agar). The spores were isolated by scraping in water and the resulting suspension was freed from solid constituents by means of Miracloth. The spore count was determined in a hematocytometer (Thoma) and brought to 10 000 spores/ml. 25 µl (accordingly 250 spores) were applied per potato disk (1.5 cm diameter) and distributed uniformly. The inoculated disks were subsequently incubated at 16°C. Scoring was done visually. The result after incubation for 14 days is shown in Fig. 6. As can be seen from the figure, the growth of the fungus on transgenic potato tubers which express sucrose isomerase is markedly reduced in comparison with the wild type.

Example 8: Generation of the plasmid pLemmi9-cwIso

To generate the plasmid pLemmi9-cwIso the promoter of the class I patatin gene B33 in plasmid pB33-cwIso was exchanged for the Lemmi9 promoter (Escobar et al. (1999) Mol Plant Microbe Interact 12:440-449), and the fusion protein of proteinase inhibitor II signal peptide and sucrose isomerase was thus placed under the control of the feeding-cell-specific promoter.

The functionality of the feeding-cell-specific Lemmi9 promoter has already been demonstrated (Escobar C et al. (1999) Mol Plant Microbe Interact 12:440-449). The plasmid pLemmi9-cwIso comprises three fragments A, B and C (see Fig. 7):

A) Fragment A comprises the tomato (Lycopersicon esculentum) Lemmi9 promoter. The fragment comprises the 1417 bp sequence upstream of the translation start (ATG) of the Lemmi9 gene and has been characterized as a functional promoter fragment (Escobar et al. (1999) Mol Plant Microbe Interact 12: 440-449, Accession Z69032). It was amplified by means of PCR from genomic tomato (Lycopersicon esculentum) DNA. The amplification was carried out using the following specific primers:

Leml: 5'atcGAATTCATAATTTAACCATCTAGAG 3' (SEQ ID NO: 28)

Lem2: 5'atcGGTACCTGCTTCTGGAACGAAAGGG 3' (SEQ ID NO: 29)

In order to clone the DNA into the expression cassette, the primers additionally have the following restriction cleavage sites: Primer Lem1, EcoRI; Primer Lem2, Asp718.

The PCR reaction mixture (100 µl) comprised:

- genomic tomato DNA (1 µg),
- primers Lem1 and Lem2 (250 ng each),
- Pfu DNA polymerase reaction buffer (10 µl, Stratagene),
- 200 µM dNTPs (dATP, dCTP, dGTP, dTTP) and
- 2.5 units Pfu DNA polymerase (Stratagene).

Before starting the amplification cycles, the mixture was heated for 5 minutes at 95°C. The polymerization steps (30 cycles) were carried out in an automatic T3 thermocycler (Biometra) with the following program: denaturation 95°C (1 minute), annealing of the primers at 56°C (40 seconds), polymerase reaction at 72°C (3 minutes). The amplicon was digested with the restriction enzymes EcoRI and Asp718 and cloned into the corresponding restriction cleavage sites of the pBluescript polylinker (Stratagene). The identity of the DNA which had been amplified was verified by sequence analysis. Thereafter, the fragment was digested with the restriction enzymes EcoRI and Asp718 and cloned into the plasmid pB33-cwIso, from which the B33 promoter had previously been deleted after partial restriction with the enzymes EcoRI and Asp718.

B) Fragment B comprises the nucleotides 923 to 1059 of the potato proteinase inhibitor II gene (Keil el al. (1986) Nucl Acids Res 14:5641-5650; Genbank Acc. No.: x04118), which are fused via a linker with the sequence ACC GAA TTG GG to the E. rhapontici sucrose isomerase gene, which comprises the nucleotides 109 to 1803. Thus, a signal peptide of a plant protein, which is required for the uptake of proteins into the ER is fused N-terminally to the sucrose isomerase sequence.

C) Fragment C comprises the polyadenylation signal of the octopine synthase gene (Dhaese et al. (1983) EMBO J 2:419-426. Accession Z37515, nucleotides 1344 to 1533).

In pLemmi9-cwIso (Lemmi9 = promoter of the tomato (Lycopersicon esculentum) Lemmi9 gene, cw = cell wall, Iso = sucrose isomerase), the coding region of the sucrose isomerase gene is under

feeding-cell-specific control, the gene product is taken up into the ER.

A control construct for expressing ß-glucuronidase (Jefferson et al. (1987) EMBO J 6:3901-3907) under the control of the Lemmi9 promoter (pLemmi9-GUS) was generated analogously.

Potato cells were transformed with the construct pLemmi9-cwIso or pLemmi9-GUS as described above by means of Agrobacterium-mediated gene transfer, and intact potato plants were regenerated.

Example 9: Generation of the plasmid pΔ0.3TobRB7-cwIso

To generate the plasmid pΔ0.3TobRB7-cwIso, the promoter of the class I patatin gene B33 in plasmid pB33-cwIso was exchanged for the Δ0.3TobRB7 promoter (Opperman et al. (1994) Science 263:221-223) and the fusion protein of proteinase inhibitor signal peptide and sucrose isomerase thus placed under feeding-cell-specific control.

The functionality of the feeding-cell-specific Δ0.3TobRB7 promoter has already been demonstrated (Opperman et al. (1994) Science 263:221-223). The plant termination signal comprises the 3' end of the polyadenylation site of the octopine synthase gene. The plasmid pΔ0.3TobRB7-cwIso comprises three fragments A, B and C (Fig. 8):

A) Fragment A comprises the Nicotiana tabacum Δ0.3TobRB7 promoter. The fragment comprises the region from -298 bp to +76 of the TobBR7 gene located and characterized as functional promoter fragment (Opperman et al. (1994) Science. 263: 221-223, Acc. No.: S45406). It was amplified by means of PCR from genomic DNA of Nicotiana tabacum var. Samsun NN. The amplification was performed using the following specific primers:

Tob1:5'-GGAATTCAGCTTATCTAAACAAAGTTTTAAATTC-3' (SEQ ID NO: 30)

Tob2: 5'-GGGTACCAGTTCTCACTAGAAAAATGCCCC-3' (SEQ ID NO: 31)

In order to clone the DNA into the expression cassette, the primers additionally have the following restriction cleavage sites: primer Tob1, EcoRI; primer Tob2, Asp718.

The PCR reaction mixture (100 µl) comprised:

- genomic tobacco DNA (1 µg),
- primers Tob1 and Tob2 (250 ng each),

- Pfu DNA polymerase reaction buffer (10 µl, Stratagene),
- 200 µM dNTPs (dATP, dCTP, dGTP, dTTP) and
- 2.5 units Pfu DNA polymerase (Stratagene).

Before starting the amplification cycles, the mixture was heated for 5 minutes at 95°C. The polymerization steps (30 cycles) were carried out in an automatic T3 thermocycler (Biometra) with the following program: denaturation 95°C (1 minute), annealing of the primers at 56°C (40 seconds), polymerase reaction at 72°C (3 minutes). The amplicon was digested with the restriction enzymes EcoRI and Asp718 and cloned into the corresponding restriction cleavage sites of the pBluescript polylinker (Stratagene). The identity of the DNA which had been amplified was verified by sequence analysis. Thereafter, the fragment was digested with the restriction enzymes EcoRI and Asp718 and cloned into the plasmid pB33-cwIso, from which the B33 promoter had previously been deleted after restriction with the enzymes EcoRI and Asp718.

B) Fragment B comprises the nucleotides 923 to 1059 of a potato proteinase inhibitor II gene (Keil el al. (1986) Nucl. Acids Res. 14:5641-5650; Genbank Acc. No.: x04118), which are fused to the E. rhapontici sucrose isomerase gene, which comprises the nucleotides 109 to 1803. Thus, a signal peptide of a plant protein, which is required for the uptake of proteins into the ER is fused N-terminally to the sucrose isomerase sequence.

C) Fragment C comprises the polyadenylation signal of the octopine synthase gene (Dhaese et al. (1983) EMBO J 2:419-426. Accession Z37515, nucleotides 1344 to 1533).

In pΔ0.3TobRB7-cwIso (Δ0.3TobRB7 = truncated promoter of the tobacco TobRB7 gene, cw = cell wall, Iso = sucrose isomerase), the coding region of the sucrose isomerase gene is under feeding-cell-specific control, the gene product is taken up into the ER.

A control construct for expressing ß-glucuronidase (Jefferson et al. (1987) EMBO J 6:3901-3907) under the control of the Δ0.3TobRB7 promoter was prepared analogously (pΔ0.3TobRB7-GUS).

Potato cells were transformed with the construct pΔ0.3TobRB7-cw I-so or pΔ0.3TobRB7-GUS as described above by means of Agrobacterium-mediated gene transfer, and potato plants were regenerated.

Example 10: Infection of the plants with nematodes

Transformed plants are verified via PCR with the aid of npt-specific primers. For the infection with nematodes, the cuttings of transgenic lines which express sucrose isomerase under the control of a feeding-cell-specific promoter are first grown on medium supplemented with kanamycin and later transferred to pots containing sterile soil. The plants are grown at 22°C (16-h-day/8-h-night). The plants are infected as follows: 3 ml of a suspension (approx. 500 J2 larvae) of root-knot nematodes (Meloidogyne species) are inoculated into the soil directly next to the stems of the plants. After 2 to 3 weeks, the plants are removed from the pots and the roots are washed. Thereafter, all of the root of each plant is examined with the aid of a stereomicroscope, and the number of root knots on the root system of transgenic plants and wild-type plants is compared.

Transgenic plants which express sucrose isomerase under the control of a feeding-cell-specific promoter show a pronounced resistance to endoparasitic root nematodes. The number of galls on the root system of these plants following infestation with nematodes is reduced significantly in comparison with untransformed plants.

Example 11: In-vitro nematode resistance test

Materials:

Plants: Potato (Solanum tuberosum L. cv. Solara)
Nematodes: Meloidogyne incognita
Medium: Modified Murashige & Skoog medium (MSm; solidified with agar) consisting of micro elements and 1/2 macro elements, including vitamins, sucrose and Diachin agar (0.7%), pH 5.8.

Plants: Sterile transgenic potato plants (Solanum tuberosum L. cv. Solara transformed with pΔ0.3TobRB7-cwIso or pLemmi9-cwIso), and corresponding transgenic control plants (Solanum tuberosum L. cv. Solara transformed with pΔ0.3TobRB7-GUS or pLemmi9-GUS) were provided in glass jars comprising several plants each. Starting from each plant, in each case three lines were generated by means of stem segments and subsequent cultivation on modified Murashige & Skoog medium (MSm; solidified with agar). Each line was planted out on a separate 9 cm Petri dish. The plants were grown for 2 to 3 weeks in a light/dark regime of 16 hours light / 8 hours dark at 25°C.

Nematode stock culture:
Nematodes were obtained from sterile stock cultures. M. incognita was grown monoxenically in the dark at 25°C on Cucumis sativus root explants as described by Wyss et al. (Wyss U et al. (1992) Nematologica 38:98-111). Egg sacks were collected from the sterile cultures and placed on a sieve in a glass funnel with sterile water. The funnels were connected to plastic tubing sealed with clamps. Hatched juveniles were obtained by opening the clamp and decanting the suspension into small vessels. The viscosity of the suspension was increased by adding a suspension of sterile "Gel Rite". The nematode density in the suspension was determined and standardized by adding sterile water.

Inoculation with nematodes
As soon as the plant roots had developed a root system, the roots were inoculated with freshly hatched juvenile second-instar nematodes (J2). Ten drops comprising in each case 10 juveniles were applied to each plant.

Evaluation:
After 2 to 3 weeks, the nematodes had penetrated the roots, and galls had formed in the control plants. Gall development was used to indicate successful penetration and the establishment of feeding sites in the roots. The roots of the various plant lines were examined for galls under the microscope, and the galls were recorded on the Petri dish.

In comparison with the control plants, the potato lines transformed with pΔ0.3TobRB7-cwIso or pLemmi9-cwIso revealed a significantly reduced gall development. This means a significant reduction in the damage caused by the nematodes.